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A MORPHOLOGICAL STUDY OF SARGASSUM FILIPENDULA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXII.

ETOILE B. SIMONS.

(WITH PLATES X AND XI)

THE family Fucaceae is less understood than its position and prominence in the Phaeophyceae warrant. Many important types have scarcely been considered at all, and, moreover, aside from the comparatively recent cytological studies in the family, few investigations have been conducted with modern methods of technique. The problems of morphology and cytology in the Fucaceae center chiefly around the sexual organs; the peculiar sunken structures in which they are borne, termed conceptacles; the likewise sunken but sterile structures called cryptostomata; and the sporelings.

The present investigation of these structures in *Sargassum filipendula* Ag., a member of perhaps the most highly differentiated genus in the Fucaceae, was undertaken with the hope of filling some of the obvious gaps in our knowledge of this family. It was conducted in the University of Chicago and at the Marine Biological Laboratory, Woods Hole, Massachusetts, under the direction of Professor BRADLEY MOORE DAVIS, who suggested the research to me. It gives me pleasure to express here, both to him and to Professor JOHN MERLE COULTER, my appreciation of valuable suggestions and assistance given me in this work. My acknowledgments are also due the Carnegie Institution for the use of a table at the Marine Biological Laboratory during the summer of 1904.

References to anatomical and morphological work which concern

this subject will be given under the topics to which they belong. The early history of the genus with its taxonomic bearing is omitted, as having no place here, but the once credited distribution of Sargassum which was convincingly disproved by KUNTZE ('81) is a matter of history which deserves at least brief mention.

KUNTZE relates that LINNAEUS believed that a vast area of sea was densely covered by Sargassum in active vegetative condition; HUMBOLDT reported that the region surpassed Germany in extent six or seven times; MAURY stated that it equaled the Mississippi valley; and HOECKEL estimated its area to be forty thousand square miles. That these views were generally accepted is well known. They led to instruction regarding a "Sargasso Sea," whose supposed limits were outlined upon maps of the world. KUNTZE, by comparing his own observations and those of other travelers over routes which crossed in different places the outlined area, was able clearly to disprove the existence of such a "sea." Sometimes a voyage was made through the mapped region and little or no Sargassum was seen, and again it appeared somewhat abundantly, but without definite limits or fixed location. Storms which sweep tropical shores, near which attached Sargassum grows abundantly, were found to be in great part accountable for the appearance of the larger quantities of floating Sargassum. KUNTZE obtained no evidence to substantiate the view that floating Sargassum vegetates. It had been believed that floating forms of Sargassum consisted of *S. baccifierum* only, but KUNTZE found several species floating, and observed that the specimens in herbaria which had been collected in mid-ocean and labeled *Sargassum baccifierum* according to general belief, could be referred to various species. He therefore concludes that there is no characteristic floating species. The appearance in mid-ocean of floating masses now and then does not seem strange when the authentic distribution and abundance of attached Sargassum are recalled. According to KJELLMAN ('93) this genus, which includes one hundred fifty species, over half the number belonging to the entire family, is found attached along the coast of all warm seas, reaches north to Cape Cod in the Atlantic, to Japan in the Pacific, and in the south into Australian waters, where it is the most abundant. With the extent of this distribution in mind the presence of floating masses, especially after storms, is to be expected.

MATERIAL AND METHODS.

Material for this study was collected near the shores of Woods Hole, late in July and during August. Plants both in vegetative and in reproductive conditions were abundant. The weak solution of chromacetic acid of Flemming (1 per cent. chromic acid 25^{cc}, 1 per cent. acetic acid 10^{cc}, water 65^{cc}) proved a satisfactory killing and fixing reagent. Microtome sections were cut from paraffin 5 μ in thickness and stained either by iron-alum-haematoxylin after the method of Heidenhain or by safranin and gentian violet. The mucilage on the surface of the plant and in young conceptacles and cryptostomata takes the anilin dyes readily, but is not especially troublesome.

GENERAL MORPHOLOGY AND HISTOLOGY.

The habit of *Sargassum filipendula* is so like that of other species which have been described that it needs but slight attention. This species grows attached to rocks below low water mark, and therefore, unlike Fucus and Ascophyllum, is never exposed to the air. Vegetative plants and reproductive plants bearing all stages of conceptacles are plentiful in summer. Sporelings are abundant also and easily collected, for the discharged eggs and their products, the sporelings, remain attached for some time by mucilage to the surface of reproductive branches near the parent conceptacles.

The stem arises from a small disk-shaped holdfast and passes into long cylindrical branches which bear spirally arranged leaves, berry-like floats, which seem to be modified portions of leaves, as generally stated, and short reproductive branches. This form may attain a height of 60^{cm}, but is commonly shorter. Cryptostomata develop upon stems, leaves, and occasionally also upon reproductive branches in Sargassum, which differs in this respect from Fucus, whose receptacles, according to BOWER, contain no cryptostomata.

KJELLMAN ('93) states that the conceptacles of Sargassum are hermaphrodite. In *Sargassum filipendula* both mature bisexual and unisexual conceptacles are formed. Some conceptacles contain only spermatocysts (antheridia); some, more rarely, contain many spermatocysts and but one or two oocysts (oogonia); and others bear only oocysts. The appearance of a conceptacle devoted to the formation of oocysts differs decidedly from such a structure in Fucus. In

Sargassum the oocyst has no stalk cell. It is an embedded organ, being almost surrounded by wall cells of the conceptacle. As both the size and contents of a conceptacle are dependent upon the activity of wall cells (as described later), this conceptacle in Sargassum is smaller and has fewer sexual organs and paraphyses than the corresponding conceptacle in Fucus. The unisexual tendency in the conceptacle of Sargassum may be due in part to the unproductiveness of the many wall cells which abut upon the embedded oocyst.

The anatomy of the thallus of Sargassum has been studied in four species. In 1876, REINKE reported its development in *Sargassum Boryanum* from a three-sided apical cell situated at the bottom of a pit in the apex of the stem. He stated that the holdfast is composed of rhizoids and that a few intercellular filaments occur in the old parts of the thallus. OLTMANNS ('89) in an anatomical investigation of *Sargassum linifolium* and *S. varians*, likewise described a three-sided apical cell, and in addition gave an account of the origin both of the apical cell of a leaf and of a branch. He believes that the branching in Sargassum holds no relation to dichotomy. He figures an enlarged epidermal cell near the apical cell of the stem, and states that it becomes a three-sided apical cell. This young cell develops an outgrowth in which a second apical cell is soon differentiated, between the first and the stem. The first formed apical cell develops a leaf and the last a branch. OLTMANNS agrees with KUNTZE ('81) that there are all gradations between leaves and floats, and that floats are modified portions of leaves.

In 1892, HANSTEEN published the results of an anatomical and physiological investigation of *Sargassum bacciferum*. He also reported a three-sided apical cell, but did not trace its origin in any structure. He described three kinds of tissues, naming them the assimilating system, the storage system, and the conducting system. The assimilating system, according to HANSTEEN, includes only the outer layer of cells, or epidermis. Its cells are twice as long as broad, have undulating walls, like the epidermal cells in higher plants, and contain "phaeoplasts." The cells of this system add to their own number by radial, and to the cells below by tangential, divisions. The storage system occupies a zone several cells wide between the assimilating system and the innermost tissue which constitutes the conducting

system. Most of the cells in the storage system are large. HANSTEEN found them empty in alcoholic material of Sargassum, but he did not doubt their function to be that of storage, because he had found much reserve material in similar cells of living Fucus. The conducting system consists of an axial cylinder of long cells with small diameter and oblique end walls. These cells are believed by HANSTEEN and others to function as sieve tubes. The cells of the three systems communicate by pores.

HANSTEEN observed in the storage cells of *Fucus serratus* and several other types, spherical grains of different sizes, which he named fucosan. He believes that the same structures have been variously considered as fat, proteid, and starch by other observers. The grains do not stain blue with iodin, and are soluble in water. HANSTEEN, who made a chemical analysis to determine their composition, considers them as a carbohydrate with the formula $(C_6H_{10}O_5)_n$. CRATO ('**92**) described in *Chaetopteris plumosa* spherical or elliptical bladder-like structures which he named physodes. He reported ('**93**) that they contain phloroglucin as a constant ingredient, function in directing the chemical exchange and transportation of food material within the cell, have motion, and are independent cell organs like the nucleus and chromatophore. CRATO stated further that HANSTEEN had confused various cell contents, and that fucosan grains and physodes are the same. KOCH ('**96**) denied the presence of phloroglucin in these bodies. In a later paper HANSTEEN (:**00**) again discusses fucosan grains. He maintains that CRATO'S physodes are fucosan grains, and that they are not independent cell organs but products of the phaeoplast. HANSTEEN has made no further chemical analyses to determine the nature of the bodies, but holds that they surely represent a product of photosynthesis. HANSEN ('**95**) after an investigation of several forms (*Dictyota dichotoma*, *Taonia atomaria*, *Halyseris polypodioides*, Asperococcus, Hydroclathrus, and Cystoseira), states that the Phaeophyceae contain oil and no starch, and OLTMANNS (:**04**) expresses the same view. It is seen therefore, that the character of the reserve material in the cells of the Phaeophyceae is still somewhat problematical.

Every stem and leaf structure in *Sargassum filipendula*, as in other species studied, develops through the activities of a three-sided apical

cell. The tissue systems described by HANSTEEN are present and each seems to have the function ascribed to it, although without rigidity. Each system, too, has its origin in the group of segments surrounding the apical cell and can be traced very near it. The cells of every system are meristematic in the apical region, but the epidermal cells are apparently the only ones which retain this activity. The cells of any one of the three systems correspond well in general appearance with the similarly placed cells described by HANSTEEN, but an interesting modification was observed in the cells of the conducting system. All are long and of small diameter, but in respect to thickness of walls the tissue is differentiated into two regions. The inner cells have thin walls, while the outer ones have thick walls. The thick-walled cells may be both supporting and conducting in function. The conducting system of a leaf blade consists only of thin-walled tissue. No intercellular filaments, as reported by REINKE, have been found. Sometimes, however, a filamentous alga creeps into the mucilaginous walls of cells near the surface of a leaf or old stem, and gives the appearance of intercellular filaments. As the little alga contains true starch, its cells when stained with iodin present a sharp contrast to the unstained cells of Sargassum. HANSTEEN ('92) figures pores in thin areas consisting of the middle lamella in *Sargassum baccifierum*, and REINKE ('76) represents similar areas but without pores in cell walls of *Fucus vesiculosus*. Such thin areas are common between cells in the tissues of *Sargassum filipendula*, but pores, though probably present, are rarely seen.

The character of the reserve material in Sargassum proved of great interest. Sections from plants which have been preserved in formalin contain much more stored material than tissues which have been kept in alcohol. Preparations, however, which have passed through alcohol, xylol, paraffin, the heat of the bath, etc., still contain within the cells of the epidermis and outer cortex, many bodies which in all probability represent reserve food material. These bodies, which stain readily, vary in size and structure, but are evidently related, for transitional stages can be found between the most extreme forms. Judging by the appearance of the structures, some are intact and others modified. Those which seem intact are spherical, with a diameter which equals or exceeds the length of a chromatophore. Each con-

sists of a more or less homogeneous ground substance and one or more refractive areas which are somewhat centrally placed. The modified structures vary from spheres, whose ground substance has been changed only at the periphery, to swollen masses which have an entirely modified ground substance with an irregular outline. Both the intact and modified bodies may occur within the same cell; but the former and the least modified are more common in epidermal cells, whereas the most modified are in cortical cells. The occurrence of such bodies within epidermal cells where photosynthesis is the most active, suggests that they represent a manufactured food. The varied modifications in the structures indicate the solvent action of the killing fluid, or an intercellular enzyme. As the inner cells contain bodies presenting greater modifications than the epidermal cells, the agent producing the change is apparently applied from within the tissue. If then within, it is probably an enzyme, for a solvent used in the process of killing would attack the contents of epidermal cells, doubtless before any others. The intact bodies may represent a newly formed product, perhaps a carbohydrate, and the modified structures, the product in process of digestion. The bodies do not stand with iodin in any condition. If they are carbohydrate they probably differ as much or more from the starch of higher plants as does inulin. The presence of many small spheres in formalin material and their absence from tissues preserved in alcohol indicates that oil globules are present in the cell, in addition to the structures described above. Future investigations on living material will probably disclose the presence of both oil and a carbohydrate in the Phaeophyceae.

THE ORIGIN AND DEVELOPMENT OF THE CONCEPTACLE.

The conceptacle in the Fucaceae had been but little studied when BOWER ('80) gave an account of its development in four genera and six species (*Fucus serratus*, *F. platycarpus*, *F. vesiculosus*, *Ozonthallia nodosa*, *Halidrys siliquosa*, and *Himanthalia lorea*). According to him the development of the conceptacle in every species conforms to one scheme with minor variations.

The "initial cell" of the conceptacle, as stated by BOWER, is the terminal cell of a linear series which is produced by a modification of the regular divisions in the segments of the apical cell of a receptacle.

This initial cell, strangely, contributes nothing essential to the conceptacle. It either degenerates directly without having divided at all, or it produces a short filament whose terminal portion degenerates. A cortical cell below the initial is termed by BOWER a "basal cell." This cell and others which adjoin the initial cell laterally, divide and form the walls of the conceptacle from which the sexual organs and paraphyses arise. The initial cell, therefore, according to BOWER, takes no part in the development of the conceptacle, whereas the cells adjacent to the initial produce all that is important, the walls and their products. The prominent features of this scheme for the development of the conceptacle are, it is seen, degeneration of an unimportant initial cell or a part of its filamentous product, and the activity of cells adjacent to the initial in producing the entire conceptacle.

Nearly all contributions in this field since 1880 have been in the main confirmatory of the work of BOWER. VALIANTE ('83) states that the development of the conceptacle in Cystoseira is due to the growth of neighboring tissue, about one or two cells. OLTMANNS ('89) describes the walls of the conceptacle of *Halidrys siliquosa*, *Himanthalia lorea*, and *Ascophyllum nodosum*, as also formed by neighboring cells, with the one exception that in Ascophyllum the initial cell develops a mass of tissue in the base of the conceptacle. This tissue, he reports, shares with the rest of the inner surface formed from neighboring tissue, in developing the sexual organs. As no degeneration of tissue was observed in Ascophyllum, and as its initial cell does contribute some important tissue the development of the conceptacle, this genus presents an exception to a part of the scheme which BOWER reports. Although Sphlachnidium should no longer be included in the Fucaceae, as shown by the Misses MITCHELL and WHITTING ('92), it is of interest to note that these investigators report its conceptacle as developing by the radial division of cells adjacent to a persistent but inconsequential element, which they believe to be homologous with the initial cell of BOWER. GRUBER ('96) states that the conceptacle of *Seirococcus axillaria* is more like that of Halidrys than Ascophyllum, which means, again, that it has an initial cell which contributes nothing of consequence to the conceptacle, whose walls are formed by cells which are adjacent to the initial.

HOLTZ (:03) reports that in the development of the conceptacle of

Pelvetia fastigiata several epidermal cells cut off basal segments which divide transversely until six or more tiers are formed. Over these tiers, one or more epidermal cells break down and a cavity results, which is gradually enlarged by further disintegration of epidermal and meristematic cells. After a time this process ceases, and a "healthy surface" is formed from the deeper meristematic cells. This surface, which comprises the walls of the conceptacle with the exception of the upper part that is formed by "cortical rows" of cells, produces sexual organs and paraphyses. The prominent features which distinguish the conceptacle of Pelvetia from others, as thus described, are the presence of several epidermal or initial cells, the more extended disintegration of tissue, and a difference in the behavior of the basal cells.

The development of the conceptacle in *Sargassum filipendula* is at variance with all the prominent characteristics in the development of the conceptacle as described by BOWER. The initial cell of Sargassum does not break down. It is an active cell which produces the entire conceptacle. As the whole conceptacle is the product of this one cell, adjacent cortical tissue takes no part whatever in the development of the structure. The first indication of the conceptacle is a clearly differentiated epidermal cell which lies near the apical cell of a reproductive branch (*fig. 1*) and constitutes the initial cell of the conceptacle. The upper portion is surrounded laterally by epidermal tissue, whereas its central and basal regions are bounded by cortical. The initial is much larger than any of the cells with which it is in contact and differs much from them in shape. Though it may vary somewhat in length it is always flask-shaped. Its oval bowl, sometimes slightly narrowed at the base, tapers above into an elongated neck whose outer end is flush with the surface. The initial cell is circular in cross section at its apex (*fig. 1a*) and elliptical at its base (*fig. 1b*).

The initial cell never breaks down. On the contrary the development of the conceptacle is initiated by its activity. Its large nucleus divides. Then a curved wall is formed with concave surface above, separating two very unlike cells (*fig. 2*), which form the two-celled stage of the conceptacle. The upper cell, which may be designated the tongue cell, is a long somewhat cylindrical structure; whereas the lower is somewhat conical or wedge-shaped. The initial cell and the two-celled stage of the conceptacle have similar outlines both in longi-

tudinal (*figs. 1* and *2*) and in transverse sections (*figs. 1a, m, b,* and *2a, m, b*). That the lower portion of the tongue cell is surrounded by the upper part of the cell below it is well shown in both longitudinal and transverse sections of the two-celled stage of the conceptacle.

The lower cell of this two-celled structure divides longitudinally into two similar daughter elements, thus producing the three-celled stage of the conceptacle (*fig. 3*). The longitudinal wall reaches to the lower portion of the tongue cell, whose basal portion is surrounded now by two cells instead of by one. The relative position of the three cells is made clearer by an examination of their transverse sections. A cross section near the base of the three-celled structure shows two similar cells (*fig. 3b*). A cross section about midway between the apex and base shows three cells (*figs. 3m* and *3bm*), the tongue cell and the two lower cells which surround its base. A section of the apex is circular in outline and consists of the tongue cell alone (*fig. 3a*). The three-celled stage of the conceptacle is apparently formed occasionally in another way. The two longitudinal sections of an initial cell are shown in *figs. 4, 5*, containing three nuclei but no walls. Two nuclei appear in one section and one in the other. It seems that the nucleus of the initial cell in this instance divided first with its spindle perpendicular to the axis of the cell, and that one of the daughter nuclei divided with its spindle parallel to the axis.

After the three-celled stage, the development of the conceptacle is readily followed. The two lower of the three cells divide longitudinally in various planes. A condition thus results which exhibits five cells in longitudinal median section (*fig. 6*). Four of the five cells are young cells of the recent divisions, and one is the centrally placed tongue cell. Longitudinal divisions continue as before, and a structure showing six or seven cells in longitudinal section is formed (*fig. 7*). The tongue cell is still conspicuous in this and in several succeeding stages. Longitudinal divisions continue as illustrated in *figs. 8, 9, 11*, until the walls of the entire conceptacle are formed. Some of the wall cells begin to develop sexual organs when the conceptacle is very small (*figs. 9, 11*). This activity of the cells, however, does not prevent them from contributing to the growth of the conceptacle. The mouth of the conceptacle is surrounded by a marginal ring of epidermal tissue about one or two cells deep (*figs. 8, 11*). As these

cells are not aggressive they may be omitted from further consideration. Excluding this minor detail every portion of the conceptacle is the product of one initial cell. Cortical tissue adjacent to the initial takes no part in its development.

The behavior of the tongue cell is similar to that of the "initial cell" in other forms as reported by BOWER. It may show signs of degeneration (*fig. 8*), may remain inactive for some time (*fig. 11*), or may even divide to form a filament of two or three cells (*figs. 9, 10*). In no case does it contribute to the walls of the conceptacle, but on the contrary after its divisions resembles a paraphysis. The tongue cell is very conspicuous until sexual organs begin to develop, but shortly after their appearance it cannot be distinguished. The upper and lower cells which result from the transverse division of the initial cell (*fig. 2*) correspond in appearance and behavior with the "initial cell" and "basal cell" as described by BOWER and others. It seems probable that BOWER saw both the initial cell and the two-celled stage of the conceptacle, but failing to observe the division in the initial cell, considered the initial and the upper cell of the two-celled stage identical. With this construction, degeneration of the upper or tongue cell was believed to be degeneration of the initial cell itself, and the division of the lower cell of the two-celled stage, a product of the initial cell, was regarded merely as the division of an unrelated cortical cell. A conceptacle developed from cells which happen to be adjacent to a degenerating and unimportant cell would be a very different structure from a conceptacle developed from one active initial cell.

THE ORIGIN AND DEVELOPMENT OF THE CRYPTOSTOMA.

The references embodied in the preceding treatment of the conceptacle constitute the chief source of information bearing upon the cryptostoma. The structure which produces the sexual organs has commonly and naturally been given first attention, but investigators who have studied both, generally agree that the conceptacle and cryptostoma are homologous. Different theories regarding the significance of the cryptostoma have been offered, but no safe generalization can be made until a more extended investigation of both structures has been made in a variety of forms.

Miss BARTON ('91) gave an account of the cryptostoma in Turbi-

naria, stating that an initial cell divides longitudinally, thus forming two daughter elements which produce paraphyses. In demonstration of this two paraphyses are figured arising from the base of a many-celled structure. Miss BARTON does not report the origin of the walls of the cryptostoma, but as the initial cell is believed to develop directly into paraphyses, we may assume that she believed the walls to arise from neighboring tissue in accordance with the views of the earlier writers.

The development of the cryptostoma in Sargassum follows step by step the history of the conceptacle. The initial cell arises near the apical cell of a leaf or vegetative branch. Longitudinal and cross sections of this cell (*figs. 12, 12a, m, b*) show the same form and structure as the longitudinal and cross sections of the initial cell of a conceptacle (*figs. 1, 1a, m, b*). The activities of the two initials are also identical. The initial cell of the cryptostoma divides transversely, forming a two-celled structure (*fig. 13*) which is comparable in every way to the two-celled stage of the conceptacle (*fig. 2*), consisting as it does of a tongue cell and a larger lower cell. The lower cell divides longitudinally. A group of three cells then results (*figs. 14, 15*) which is entirely similar to the three-celled stage of a conceptacle (*fig. 3*). The two lower cells of this three-celled stage then divide longitudinally in one or more planes, forming a structure which shows four or five cells in longitudinal section (*figs. 6, 17*). The center of this structure and of several which follow is occupied by the conspicuous tongue cell (*figs. 16, 17, 18, 19*). Thus by the continued longitudinal divisions of the products of the lower cell of the two-celled stage, the walls of the entire structure are gradually developed. Paraphyses begin to appear in the cryptostoma (*figs. 18, 20*) as early as do the sexual organs in the conceptacle (*fig. 9*). The activity of the wall cells in producing paraphyses, however, does not interfere with their functioning further in developing the cryptostoma (*fig. 21*). Epidermal cells at the mouth of the cryptostoma form here, as in the conceptacle, a marginal ring one or two cells deep (compare *figs. 8* and *19*). The origin of the true walls of the structure, however, may be traced as in the conceptacle to the lower cell resulting from the transverse division of the initial.

The behavior of the tongue cell in the cryptostoma is similar to

that of the corresponding element in the conceptacle. Occasionally the tongue cell of the cryptostoma may develop a prominent filament (*fig. 20*), which is clearly identical in structure with a typical paraphysis (*fig. 21*). The young conceptacle and cryptostoma are so alike that they can only be distinguished by their respective positions on fruiting branches or on young vegetative structures, until the appearance of sexual organs in the one and paraphyses in the other defines their mature characters.

The development of the paraphysis is interesting for its regularity. A wall cell enlarges, pushing into the cavity of the cryptostoma, and then divides transversely (*figs. 18, 20*). The upper cell produces the paraphysis, whereas the lower functions in the development of the wall. The growth of the paraphysis results from the transverse divisions of the cell next the wall (*figs. 21, 22*), a method of growth termed *trichothallic*. The development of a paraphysis in the cryptostoma of Sargassum is, therefore, characteristically basipetal, as BARTON ('91) found in Turbinaria.

A somewhat advanced paraphysis is composed of three regions. That which adjoins the wall of the cryptostoma consists of the large turgescent meristematically active basal cell (*fig. 22*). The middle region is occupied by six or eight short cells which have so recently been formed that they have not had time to lengthen much. The upper region contains several greatly elongated cells. This region in a mature paraphysis is partly within the cryptostoma and partly without, for fully developed paraphyses extend far beyond the surface of the plant.

A peculiar condition found in many cryptostomata deserves special attention. Structures frequently appear between the paraphyses which seem to bear no relation to them. These are papillae and stalked cells, the former like the papillae which precede male organs in a conceptacle and the latter like the male organs themselves. The stalked cells, although slender and probably always sterile, appear to be spermatocysts no longer functional. This surprising condition is of great interest and importance in relation to the homology and significance of the cryptostoma, a structure formerly believed to contain only paraphyses, but which appears also to have sexual organs or their degenerate representatives. That the cryptostoma and conceptacle

are homologous cannot be doubted, since their origin and early development are identical in all details. The occasional appearance of sterile representatives of sexual organs within the cryptostoma further confirms this view of their relationship and strongly supports the theory of BOWER ('80) that the cryptostoma in the Fucaceae is derived from the conceptacle.

The occurrence of conceptacles in special reproductive branches only, the appearance of cryptostomata in both vegetative and reproductive branches, and the development of representatives of sexual organs within the cryptostomata, suggest a line of evolution from plants bearing conceptacles scattered over leaf and branch structures indiscriminately, to the type now under consideration with localization of the conceptacle upon special branches. Certain branches were set apart to bear conceptacles as the conceptacles in all other parts of the plant body were rendered sterile and thus changed into cryptomostata. The presence of sexual organs or their degenerate representatives within a cryptostoma indicates, according to these views, that the process is not carried to its farthest point in Sargassum.

The production of conceptacles upon small special branches only, instead of upon the entire plant, naturally results in fewer conceptacles upon one plant. The conceptacles, however, are much more closely placed than the cryptostomata. On account of their comparatively small size the initials and young conceptacles occupy very little space in the apex of a branch, but farther down on the receptacle the bulging bowls of the developing flask-shaped conceptacles require more and more space, until the mature structures nearly fill the interior of the receptacle and there is only enough intervening tissue to hold the conceptacles together. The cryptostomata, on the other hand, are well scattered upon vegetative branches and mature leaves. The contrast in the placement of cryptostomata with that of conceptacles is, therefore, very marked.

THE SPERMATOCYST.

The male sexual organs (antheridia), which will be called spermatocysts in this paper, according to the terminology of DAVIS (:04), develop from wall cells of the conceptacle in Sargassum as in other forms of the Fucaceae. A wall cell puts forth a papilla (*fig. 23*) which

is cut off by a transverse wall (*fig. 24*). The lower cell becomes a part of the wall occupying the place of the cell from which it arose. The upper cell enlarges for a time and then divides, forming the sperm mother-cell or spermatocyst and its stalk (*fig. 24*, at the right.) A stalk cell may have no other relation than that which it bears to the spermatocyst which it supports, or it may function in other ways. It may produce several spermatocysts directly, without individual stalks; it may put forth a papilla which gives rise to a spermatocyst and stalk (*figs. 25, 26*); or it may develop a hair (*fig. 27*). Hairs, however, are comparatively rare within a conceptacle of Sargassum. Owing to the variety of activities which belong to a stalk cell, the growths within a conceptacle lack uniformity. Some structures reach but a little distance above the wall of the conceptacle, whereas others form conspicuous branch systems. Although these systems are prominent in this conceptacle, they are considerably smaller and less dense than the branch systems in a conceptacle of Fucus, and there is far more unoccupied space within the cavity of a conceptacle of Sargassum than of Fucus.

The young spermatocyst contains dense cytoplasm, a centrally placed nucleus and deeply staining granules, the nucleus remaining in a resting condition for a long period. The divisions of the nucleus were not studied in detail. Sixty-four sperms are apparently formed (*figs. 27* and *28*), agreeing, therefore, with the count announced by BEHRENS ('86) for *Fucus vesiculosus*. The sperms within the spermatocyst have an elliptical outline, a cytoplasmic ground mass, and a somewhat spirally arranged band, which is probably the nucleus. The discharge of sperms was not seen, but a rent, partly terminal and partly lateral in empty spermatocysts, indicates their mode of escape.

THE OOCYST.

The female sexual organ (oogonium) or oocyst, according to the terminology of DAVIS (:04), is peculiar among the Fucaceae, as far as is known, in that it is not borne upon a stalk but is a partially embedded organ (*fig. 31*). The sister cell of the oocyst, instead of developing into a pedicel cell as is usual in this family, functions as one of the wall cells of the conceptacle. The oocyst enlarges greatly, but becomes nearly surrounded by adjacent wall cells.

Its development is simple. A somewhat enlarged wall cell of a young conceptacle divides transversely, forming two cells much alike in size and contents (*fig. 29*). The inner cell, which is the homologue of the stalk cell of the female organ in Fucus, cannot be distinguished from neighboring wall cells shortly after its formation. The outer cell, which has a free surface toward the interior of the conceptacle, increases greatly in size and soon becomes the spherical oocyst. *Fig. 30* represents a young oocyst and its sister cell, already unequal in size. There now follows a long period of growth, during which the oocyst attains a remarkable size, finally containing a great quantity of reserve material, many chromatophores, much cytoplasm, and a large nucleus. The mature organ, drawn under a lower magnification than *fig. 30*, is represented in *fig. 31*. No trace of its sister cell could be found.

The oocyst of Sargassum develops but one egg. The mitosis within the wall cell whose division produces the oocyst is normally the only mitosis in the process of oogenesis. Particular attention was given to this point. The one nucleus of the oocyst remains in a resting condition throughout the entire period of the growth of the cell, and therefore becomes the nucleus of the egg. In the other genera of the Fucaceae, as is well known, there are three mitoses within the oocyst, resulting in eight nuclei. Each of the eight nuclei may become a center for the development of an egg as in Fucus, or some nuclei may degenerate and a less number of eggs be formed, as in Ascophyllum and Pelvetia. It might be supposed from these conditions in the Fucaceae that the oocyst of Sargassum would show similar nuclear divisions and degeneration, but this is not the case. The mitoses characteristic of oogenesis in Fucus are normally suppressed in Sargassum. The tendency in the Fucaceae to reduce the number of eggs produced by an oocyst reaches its culmination, therefore, in Sargassum.

It is interesting to note that Sargassum still gives proof that it belongs to the reduction series which has its beginning in Fucus and allied forms that produce eight eggs in an oocyst. Out of the great number of conceptacles examined, one oocyst was formed which contained two eggs, and two oocysts which contained eight. The oocyst with two eggs was formed in an immature conceptacle that held five normal oocysts. The two eggs appeared fairly vigorous. One of the

two oocysts which contained eight eggs was an old conceptacle, from which other sexual elements had apparently long been discharged. The eight together were smaller than one normal mature egg. The other oocyst which contained eight eggs shared a conceptacle with two normal oocysts. It was attached in the side of a conceptacle near the surface of the plant, which for a slight distance was modified in structure as if in response to an injury. It is possible in this case that the wound incited the reversion. The appearance of an oocyst containing more than one egg in Sargassum must be regarded as a rare reversion to the Fucus type.

The resting nucleus of the oocyst is always large, but varies in structure. Sometimes it has few granules and no conspicuous reticulum, whereas at other times it contains many granules and a dense network. The nucleolus is also large in size and variable in structure. At the present time no suggestion can be made to account for the changes in nuclear structure, excepting that they are the concomitants of growth and varying nutritive conditions.

The method of discharge of the egg from the conceptacle of Sargassum is somewhat unlike that reported in Fucus and other genera. In Fucus the outer membrane of the oocyst remains attached to the conceptacle, as explained by THURET, and the eggs escape in a group surrounded by a very delicate inner membrane. In Sargassum the entire oocyst becomes freed from the conceptacle and escapes. In Fucus the inner membrane dissolves or breaks, thereby freeing the naked eggs which it has enclosed. In Sargassum the wall of the oocyst swells, stretches, and sometimes ruptures, but it may persist for a long time, even enveloping later a many-celled sporeling formed within it. The inner membrane enclosing the eggs of Pelvetia is separated from the outer as in Fucus. In Pelvetia, however, as figured by THURET, this membrane persists about the eggs, apparently offering no great resistance to the entrance of sperms. Whether the sperm enters the egg of Sargassum through a break in the oocyst membrane, whether it passes through the membrane, or whether the eggs develop parthenogenetically, is not known. A study of fertilization in Sargassum is surrounded by serious technical difficulties because both eggs and sperms develop upon the same plant, thus making it difficult to isolate the sexual cell.

THE SPORELING.

Many if not all of the eggs of Sargassum on leaving the conceptacle become fastened immediately by the mucilaginous wall of the oocyst, which still surrounds it, to the surface of the reproductive branch. In this position the eggs segment. The first division of the egg in Sargassum does not differentiate a rhizoidal region, as in Fucus and Ascophyllum. Instead, a many-celled ellipsoidal structure is formed, the divisions occurring with mathematical precision. Rhizoids then develop at one end with no apparent relation to a substratum or to gravity, so far as could be observed in fixed material. Sporelings sometimes occur at opposite sides of a branch with rhizoids directed toward the stem, thus showing no relation in the development of rhizoids to gravity; and again, sporelings occur with rhizoids directed away from the branch in various directions, indicating that the parent plant exerts no special influence. It is possible that the attachment of a sporeling upon a plant is so insecure that the direction of its axis may be shifted in the manipulation of material. Otherwise it is difficult to account for the conditions which were observed.

When the many-celled sporeling has reached the condition for rhizoid formation the cells at one pole elongate, thereby giving rise to a tuft of rhizoids of approximately equal length. This mass of rhizoidal filaments finally produces the characteristic disk-shaped holdfast of the mature plant. *Fig. 32* shows a sporeling in about the oldest condition in which it remains attached to the parent plant. No apical cells were found in these sporelings and therefore its differentiation must occur after the sporeling has separated from the parent plant.

The germination of the oospore deserves careful cytological investigation. Many preparations have been made and studied, but further attention will be given the subject before the observations are published. A few conditions may be noted, however. There are numerous radiations at the poles of the early spindles. The asters contain granular inclusions which suggest centrosomes, although their origin and relation to the processes of mitosis have not been traced. Walls following the mitoses are developed somewhat slowly, being formed in part at least by the membranes of contiguous vacuoles.

SUMMARY.

Each stem, branch, and leaf structure develops through the activities of a three-sided apical cell.

The thallus consists of three compact tissues, called for convenience the epidermal, cortical, and conducting tissues. The latter consists of only thin-walled cells in the leaves, but in mature stems contains both thick and thin-walled elements. A ring of thick-walled cells, which may have both a mechanical and conducting function, surrounds the thin-walled conducting cells in the center of the axis.

The tissues normally contain much reserve material, a part of which is oil, and a part, whose nature is undetermined, appears to be a carbohydrate.

Both the conceptacles and cryptostomata originate in a single flask-shaped initial cell which develops the entire structure.

The first division of the initial cell results in two unlike segments: a large lower cell which develops the walls of the conceptacle and cryptostoma; and an upper cell, the tongue cell, which either remains inactive, divides to form a short filament, or degenerates. The "initial cell" of BOWER is apparently the tongue cell, a product of the true initial cell.

The conceptacle and cryptostoma are undoubtedly homologous structures. Every stage of development in both structures is the same, from the appearance of the similar initial cells to the development of paraphyses in the cryptostomata and sexual organs in the conceptacle.

The paraphyses are developed basipetally by the division of the lowermost cell in each structure.

Spermatocysts or their degenerate representatives occur in some cryptostomata. Such conditions indicate that the cryptostomata have been derived from conceptacles whose sexual organs have become sterile.

The spermatocysts develop as in other Fucaceae, each finally producing sixty-four sperms which are discharged from a partly terminal and partly lateral rent.

The sister cell of an oocyst does not become a stalk and consequently the oocyst is an embedded structure.

The oocyst normally gives rise to but one egg. The nucleus of the oocyst accordingly becomes the nucleus of the egg.

The oocysts were found containing eight eggs each. These must be considered a rare reversion to the Fucus type.

The entire oocyst of Sargassum, unlike other genera of the Fucaceae which have been studied, is discharged with its enclosed egg. The oocyst wall may break, partially freeing the egg, or it may persist even enveloping a many-celled sporeling.

Segmentation of the egg takes place while it is attached to the surface of the plant by the mucilaginous wall which surrounds it. This segmentation results first in a many-celled undifferentiated ellipsoidal sporeling. Rhizoids develop late at one end of the multicellular sporeling, with no apparent relation to gravity or other stimulus.

Asters, containing granular inclusions suggesting centrosomes, appear at the poles of the spindles in the early mitoses of the segmentation of the egg.

THE UNIVERSITY OF CHICAGO.

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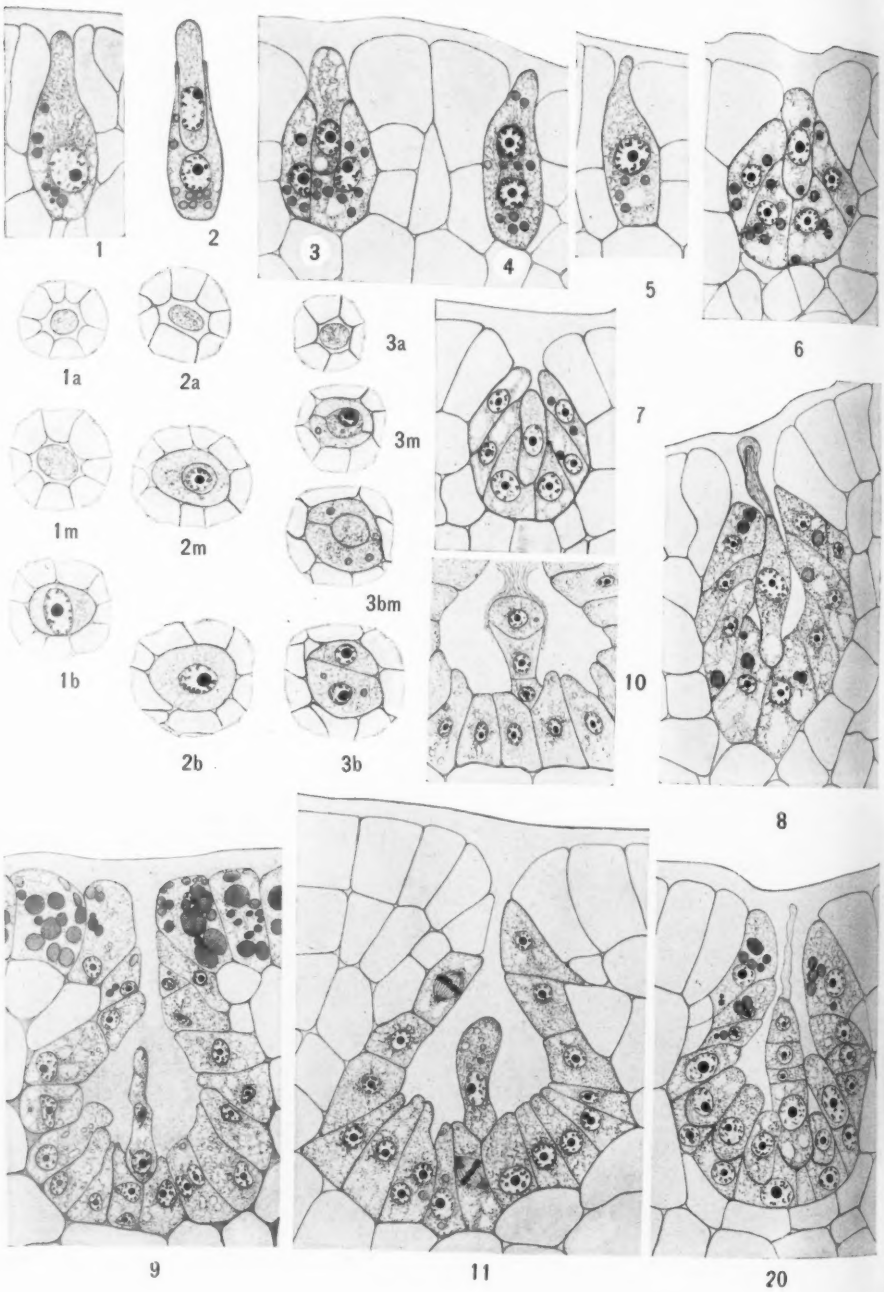
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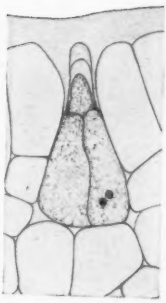

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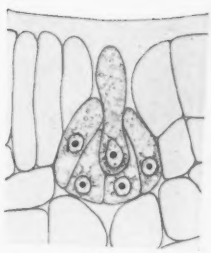

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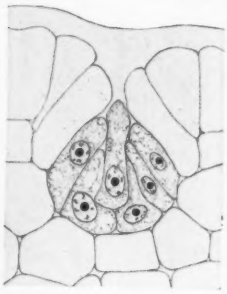

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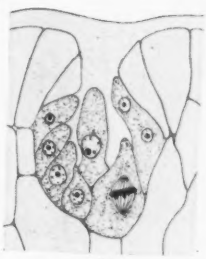

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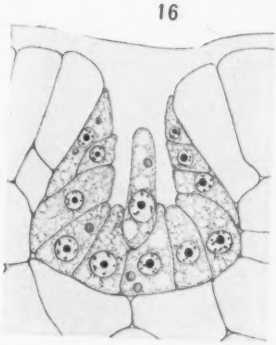

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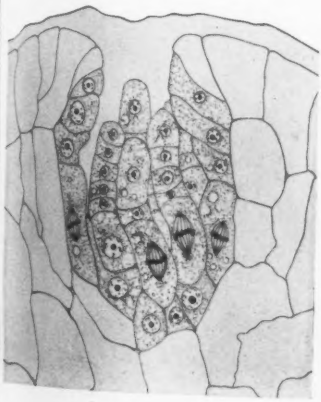

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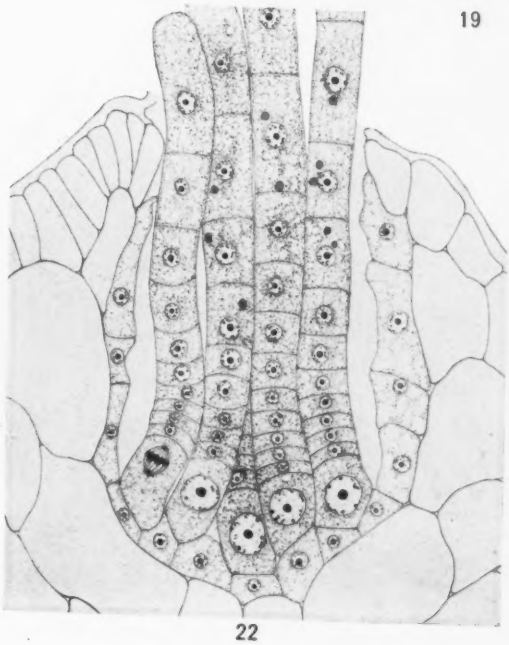

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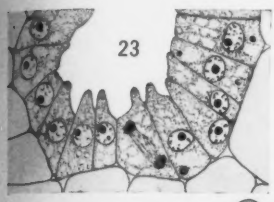

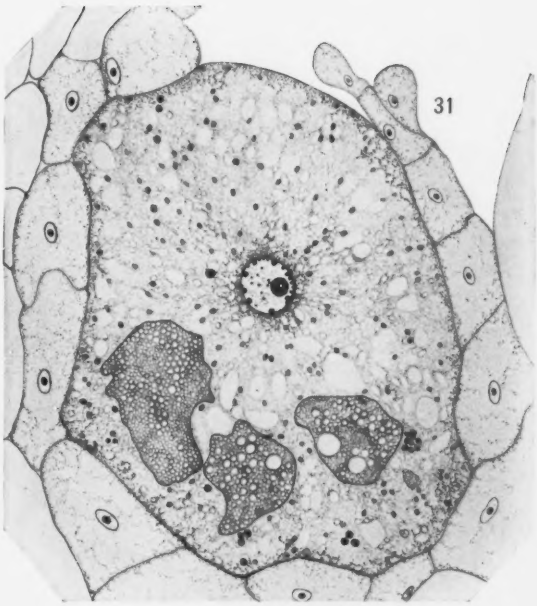

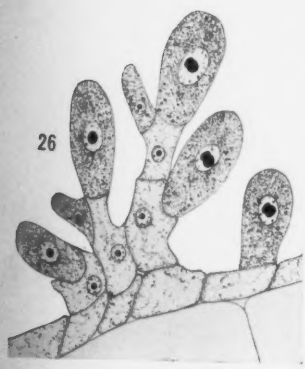

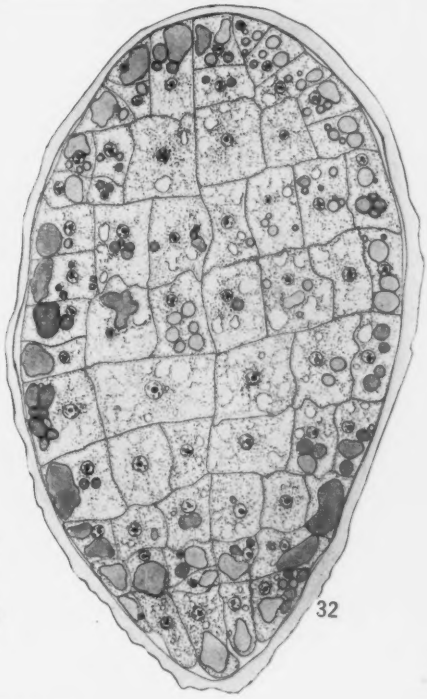

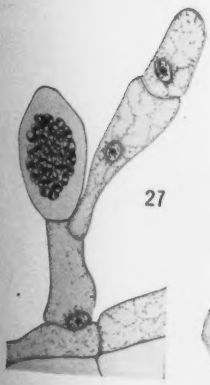

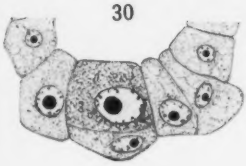

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EXPLANATION OF PLATES X AND XI.

All figures were sketched with a camera and reduced one third in reproduction. *Figs. 1–30* were drawn with Zeiss apochromatic objective 1.5^{mm} and compensating ocular number 4, magnification 1140. *Figs. 31* and *32* were drawn with dry objective, magnification 570.

PLATE X.

Figures 1–11. Development of the conceptacle.

FIG. 1. Initial cells, longitudinal section; *1a*, cross section of apex; *1m*, cross section of median portion; *1b*, cross section of basal portion.

FIG. 2. Two-celled stage, longitudinal section showing the slender upper tongue cell, and a larger lower cell; *2a*, cross section of the apex showing the tongue cell only; *2m*, cross section of the median portion with the centrally placed basal region of the tongue cell surrounded by the upper part of the lower cell; *2b*, cross section of the basal portion showing the lower cell only.

FIG. 3. Three-celled stage, longitudinal section; *3a*, cross section of the apex showing tongue cell only; *3m*, cross section of median portion with the centrally placed lower part of the tongue cell, surrounded by the upper part of its two companion cells; *3bm*, cross section a little below *3m*, showing the same cells; *3b*, cross section of the basal portion showing the two lower cells only.

FIGS. 4 and 5. Longitudinal sections of a peculiar trinucleate stage of one conceptacle. The first division of the nucleus of the initial cell must have been with the axis of the spindle perpendicular to that of the cell. *Fig. 5* contains one of the nuclei of the first mitosis and *fig. 4* the products of a division, now in late telophase, of the other nucleus of the first mitosis.

FIG. 6. Longitudinal section of a young conceptacle showing four walls cell and the tongue cell.

FIG. 7. Longitudinal section of a later stage with six similar wall cells and the centrally placed tongue cell.

FIG. 8. Longitudinal section of a more advanced stage illustrating the formation of the cavity of the conceptacle.

FIG. 9. Longitudinal section of a young conceptacle some of whose wall cells are developing papillae. The tongue cell contains two nuclei.

FIG. 10. A filament of three cells formed from the tongue cell.

FIG. 11. Young conceptacle showing simultaneous development of wall cells and papillae.

Figures 12–22. Development of the cryptostoma.

FIG. 12. Initials, longitudinal section; 12*a*, cross section of the apex; 12*m*, cross section of median portion; 12*b*, cross section of basal portion.

FIG. 13. Longitudinal section of the two-celled stage.

FIG. 14. Longitudinal section of the lateral surface of the three-celled stage.

FIG. 15. Longitudinal section of the interior of the same group of cells represented in *fig. 14*.

FIG. 16. Longitudinal section showing four wall cells and the tongue cell.

FIG. 17. Longitudinal section slightly more advanced.

FIG. 18. Longitudinal section of a young cryptostoma beginning to form paraphyses very early.

FIG. 19. Longitudinal section of an older stage which has not yet begun to develop paraphyses.

FIG. 20. Longitudinal section showing five paraphyses developing from wall cells and one from the tongue cell.

FIG. 21. More advanced, illustrating the simultaneous development of paraphyses and wall cells.

FIG. 22. Still more advanced.

PLATE XI.

Fig. 23. The development of papillae which will later give rise to spermatocysts.

FIG. 24. At the left a cell which results from the separation of a papilla from a wall cell. At the right a spermatocyst and stalk which have been formed by the division of a cell similar to the one shown at the left.

FIG. 25. A stalk cell has given rise to a papilla, now separated by a wall.

FIG. 26. A branch system formed through the activity of stalk cells.

FIG. 27. A spermatocyst containing sperms. The stalk cell has developed a hair.

FIG. 28. A mature spermatocyst, the stalk cell pushing out at one side.

FIG. 29. Very young oocyst with its sister cell, which is the homologue of the stalk cell in Fucus.

FIG. 30. Slightly older oocyst and its sister cell already unequal in size.

FIG. 31. A mature embedded oocyst containing many chromatophores and much reserve material.

FIG. 32. A sporeling still attached to the surface of the parent plant. At one pole rhizoids have begun to develop. The old wall of the oocyst surrounds the sporeling.

CHROMOSOME REDUCTION IN THE MICROSPOROCYTES OF LILIUM TIGRINUM.[1]

JOHN H. SCHAFFNER.

(WITH PLATES XII AND XIII)

THE progress recently made in our knowledge of hybrids has given a new impetus to the study of chromosome reduction. Unfortunately, there is still much disagreement in the accounts of various observers. In order to continue my own investigations on a very favorable object, the microsporocytes of *Lilium tigrinum* were selected, since material is easily obtained in large quantities. CHAMBERLAIN has studied the pollen grain of this plant and has also given figures of the microsporocyte in the spirem stage. The chromatin granules are exceptionally distinct, and this facilitates the correct interpretation of the complex figures to be seen in the reduction karyokinesis.

Recently papers on the reduction division have been published by FARMER and MOORE, STRASBURGER, MONTGOMERY, WALLACE, and others, which are in essential agreement with the interpretations of DIXON on *Lilium longiflorum* and my own observations on *Lilium Philadelphicum* and Erythronium. On the other hand BERGHS, GRÉGOIRE, and ALLEN have come to somewhat different conclusions. The observations of ROSENBERG on Drosera have opened up an important field of investigation on the individuality of the chromosome. These papers have been reviewed so recently by various writers that it is needless to discuss the results here. It is sufficient to say that it must appear to an impartial judge that the cytologist is at present able to see in his preparations almost anything which may be conceived of as taking place in the structures investigated. This, however, should not hinder work in such an important field, for the proper interpretation can be attained only by continued observation. Little can be regarded as certain until there is a more general agreement among competent investigators. So far as the present research is concerned, the extent and variety of the preparations

[1] Contributions from the Botanical Laboratory of Ohio State University. XXIV.

studied seem to preclude the possibility of mistake. Such doubts as were expressed in my former papers have been practically removed. So far as the writer is concerned, the interpretation given below is conclusive. Another investigator might perhaps come to different conclusions by using other methods.

MATERIALS AND METHODS.

Stamens of various ages were collected in Clay County, Kansas, during July 1904; killed in weak chrom-acetic acid (chromic acid 0.3^{gm}, glacial acetic acid 0.7^{cc}, water 99^{cc}), passed gradually through the grades and preserved in 70 per cent. alcohol, imbedded in paraffin, cut 10–18 μ thick, and stained on the slide. Some old slides, the material of which had been killed in the ordinary chrom-acetic acid solution, were also at hand. Various stains and combinations were used, but for bringing out the chromatin network and chromatin granules of the early stages, Delafield's haematoxylin, when properly developed, gave by far the best results, being superior in this respect to either Heidenhain's iron-alum-haematoxylin or safranin and gentian violet. Nucleoli, both in the nuclear cavity and in the cytoplasm, are stained very distinctly by the safranin-gentian-violet combination, but are only slightly affected by Delafield's haematoxylin.

I am indebted to my wife, MABEL SCHAFFNER, for the preparation of most of the two hundred serial slides on which the present paper is based.

INVESTIGATION.

Before the microsporocytes are beginning to separate the prominent chromatin network is being transformed into slender delicate threads. These threads appear to be discontinuous in some places. However, the appearance may be due to injury during the process of cutting. The threads are small in diameter as compared with the single chain of spherical chromatin granules (*figs. 1–3*). After the spirem becomes fully developed it shows no free ends and is much wound, looped, and twisted. In this stage it appears to be entirely free in the nuclear cavity and is usually in the so-called synapsis stage. Sometimes the contraction is to one side of the nuclear cavity, sometimes near the center, but often little or no contraction is evident. Whether this is an artifact or a real stage in the process of karyokin-

esis appears still to be an open question. Such contractions are so easily produced by the ordinary killing reagents used, and have been described for such a variety of the early stages of nuclear division, that it seems to me no importance is to be attached to observations which have been made on killed material. So far as any opinion is to be expressed upon the appearances in *Lilium tigrinum*, I am still convinced that the extraordinary distortions commonly figured owe their origin to the action of the fluids used before imbedding in paraffin.

The spirem remains simple during the entire synapsis. The linin thread becomes thickened and the chromatin granules are usually more or less elongated (*figs. 4, 5*). At this stage the spirem has already a strong tendency to be thrown into loops and coils (*fig. 4*). After the microsporocytes have become partly separated and more spherical, they were rarely observed to be in synapsis. The nuclear cavity enlarges and the chromatin ribbon becomes thicker, with the granules still more prominent (*figs. 6–8*). This is an important point to consider, for we have here a clear case of sporocytes, long past the supposed synapsis stage, showing with remarkable clearness a simple continuous spirem, with a single chain of chromatin granules.

Synapsis, therefore, can have nothing to do in this case with a supposed longitudinal conjugation of two spirems or two networks of chromatin before the spirem is formed. After the nuclei have passed on to the stage represented in *fig. 6*, the stages are so easily followed and the threads so prominent, that a longitudinal conjugation, if one occurred, could not escape notice. Shortly after the stage shown in *fig. 6* the spirem begins to show double rows of elongated chromatin granules, but the relative quantity of chromatin ribbon present in the nuclear cavity is not much diminished (*figs. 9, 10*). If the amount of spirem were diminished one-half by a longitudinal conjugation the fact would certainly be noticeable. Often a part of the spirem appears still single, or it will appear with a double row of granules and gradually change to a row apparently single (*figs. 11–17*). The appearance would be the same whether the granules were dividing or conjugating. The uniformity of the pairs of granules on the linin thread is remarkable, and the pairs themselves suggest a division. If a conjugation of the chromatin granules were established, it would certainly show

an almost inconceivable regularity. The granules would have to be definite and fixed, of the same number in both the egg and sperm, and not be subject to increase or diminution (*figs. 14–16*). In the early stages of the spirem there are, of course, numerous instances of threads lying parallel, side by side, but these appearances are equally common after the chromatin granules appear double (*figs. 15, 17*).

The ribbon now begins to show an arrangement into definite loops (*fig. 10*). It becomes much shorter and thicker (*figs. 18, 19*) and finally shows a definite twisting together into twelve loops, which have their heads toward the nuclear wall (*fig. 20*). At this stage the chromatin granules can still be distinguished lying side by side (*fig. 21*). But at this time the whole ribbon begins to undergo a change, so that it stains of a uniform, dense color throughout, and before the loops separate all evidence of chromatin granules is lost (*figs. 22, 23*). That the loops shown in *figs. 18–23* are the incipient chromosomes is self-evident. By no manner of interpretation can such a conclusion be explained away. By the time the twelve loops have separated, the nucleoli have entirely disappeared from the nuclear cavity (*fig. 24*). The nucleoli break up into micronucleoli and are thrown out into the cytoplasm (*figs. 38, 51*). The figures in which they do not appear were taken from material stained in such a way that the nucleoli were not evident or not very distinct.

The chromosomes are exceedingly interesting on account of the many fantastic shapes produced by the coiling of the ribbon. A series of distinct shapes is given in *figs. 25–37*. Occasionally the loop shows evidence of its double nature (*fig. 29*), but usually it appears homogeneous throughout. Much time was spent in a study of these chromosomes, and the variety of shape and coil could be extended indefinitely. In all cases the chromosome is continuous, the outer end of the loop always being closed. Occasionally the coiling takes place in such a way as to form a double loop (*figs. 26, 27, 35*). The chromosome is situated on the spindle with its head or closed end outward (*figs. 28–30*). Sometimes it is very difficult to unravel the nature of the coil, as in *fig. 35*. In other chromosomes it is an easy matter to follow out the details of the loop, as in *fig. 28*.

The spindle threads are evidently attached some distance back of the free limbs of the loop (*figs. 25–28*). The limbs are gradually

uncoiled and pulled apart. They are separated at the closed outer end. A transverse splitting of the chromosome is thus accomplished. There is no evidence that the two limbs of the chromosome are a male and female chromosome joined end to end and twisted together until they make a longitudinal pair. But theoretically such a proposition is easily possible or even probable. Because the spindle threads are attached some distance from the ends of the limbs, the daughter chromosomes are developed as V- or U-shaped loops (*figs. 40, 43, 51*). In favorably stained sections some evidence of chromatin granules may be observed. These still show a distinct pairing in some cases (*fig. 44*), but in others the arrangement is considerably disturbed (*fig. 45*). The daughter chromosomes, as they appear in the daughter star, are of various forms and are sometimes twisted (*figs. 44–50*).

The micronucleoli are gradually collected below the two daughter skeins, and are finally all inclosed in the nuclear cavities of the daughter nuclei (*figs. 52, 53*). The daughter chromosomes do not appear to form a very definite resting network, but are transformed into the mother skein of the second division rather rapidly. Whether a continuous ribbon is formed was not ascertained. The loops are already separate at an early stage, and it is possible that the daughter chromosomes of the first division, after forming an imperfect reticulum, break up directly into the twelve chromosomes of the second division (*fig. 54*). This point, however, is doubtful. But the absence of a definite resting stage in sections having microsporocytes with loose daughter skeins in close proximity to loose mother skeins of the second division, gives support to the above supposition. The micronucleoli are again distributed in the cytoplasm before the mother-star stage of the second division (*figs. 54, 55*). The karyokinetic figures of the second division are easily distinguishable from those of the first. This is especially true of the mother star (*figs. 38, 55*). The chromosomes in the second division have their free ends directed outwards, as in an ordinary vegetative division. Commonly they are more or less tangential to the spindle. Sometimes, however, they stand at right angles, as represented in *figs. 56, 57*.

One of the most difficult points to determine was the nature of the splitting in the second division. However, it was definitely ascertained that the splitting is longitudinal. Dividing chromosomes are

represented in *figs. 58–61*. The daughter loops are completely separated very early in their migration towards the poles (*fig. 62*). They are also of various shapes. Frequently straight chromosomes are present in the daughter star and very commonly the J shape predominates (*figs. 63, 64*). U and V shapes are also present. After the chromosomes have passed into the daughter skein stage they show an irregular outline with a row of irregular chromatin granules (*figs. 65, 66*). The chromosomes develop into irregular networks, showing the remains of the original loops, and thus pass into the spore tetrad stage (*fig. 67*).

During the early germination stages of the microspores the figures are again remarkable for the large nucleoli in the cytoplasm (*figs. 68–70*). From the appearance of the nuclei during the several divisions, it is evident that the nucleoli do not contribute directly to the formation of the chromosomes, but that they are uniformly thrown out into the cytoplasm, in a fragmented condition, during the earlier stages of karyokinesis.

No study of the achromatic structures was attempted, and though some interesting points were observed from time to time, there was nothing which calls for special mention.

SUMMARY.

1. The first division of the microsporocyte is a true reduction division.

2. A continuous spirem is formed with a single row of chromatin granules.

3. The spirem passes through and comes out of synapsis without a conjugation or division of chromatin granules.

4. The chromatin granules divide but the linin thread does not show a distinct separation.

5. The continuous spirem shortens and thickens and twists up into twelve loops, which are the incepts of the twelve separate chromosomes.

6. The chromosomes are arranged in the mother star with the loop or head end turned outwards and the spindle threads are attached near the ends of the free limbs or about half way between the free ends and the head.

7. During the metakinesis stage the chromosomes uncoil and separate by a transverse division at the middle.

8. The chromosomes of the second division appear to represent the daughter chromosomes of the first division.

9. The division of the chromosomes in the second nuclear division is longitudinal.

10. The nucleoli fragment and pass out into the cytoplasm during the first and second divisions and also during the germination of the microspore.

OHIO STATE UNIVERSITY,
Columbus.

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EXPLANATION OF PLATES XII AND XIII.

The plates are reduced five-eighths in reproduction. The figures representing entire cells and nuclei were studied with a Leitz no. 4 ocular and $\frac{1}{16}$ oil immersion objective; the others with a Zeiss no. 12 ocular and a Leitz $\frac{1}{16}$ oil immersion objective.

PLATE XII.

FIG. 1. Microsporocyte with chromatin network beginning to form the spirem.

FIG. 2. Microsporocyte with delicate threads showing a single row of prominent chromatin granules.

FIG. 3. Single thread of the same.

FIG. 4. Sporocytes beginning to separate. Nucleus with distinct continuous, contracted spirem in the so-called synapsis stage.

FIG. 5. Single thread of the same showing a single row of chromatin granules.

FIG. 6. Sporocyte some time after the synapsis stage, showing prominent spirem with single row of chromatin granules. The free ends are cut.

FIGS. 7, 8. Single threads of the same.

FIG. 9. Sporocyte with chromatin granules mostly double and flattened in appearance.

FIG. 10. Somewhat later stage, showing the beginning of looping of spirem, and division of chromatin granules. One nucleolus beyond the nuclear cavity.

FIGS. 11–17. Single threads from the same stage, showing chromatin granules still single, some with granules partly double, and others with typical double rows of granules.

FIG. 18. Microsporocyte with loops nearly developed.

FIG. 19. A single loop of the same stage.

FIG. 20. Chromatin loops some time before separation; somewhat cut.

FIG. 21. A single loop from the same stage.

FIG. 22. Section of microsporocyte, showing chromatin loops near the time of separation.

FIG. 23. A single loop from the same nucleus.

FIG. 24. Microsporocyte, showing the twelve chromosomes.

FIGS. 25–37. Individual chromosomes, showing various types of loops and coils and their position on the spindle threads.

PLATE XIII.

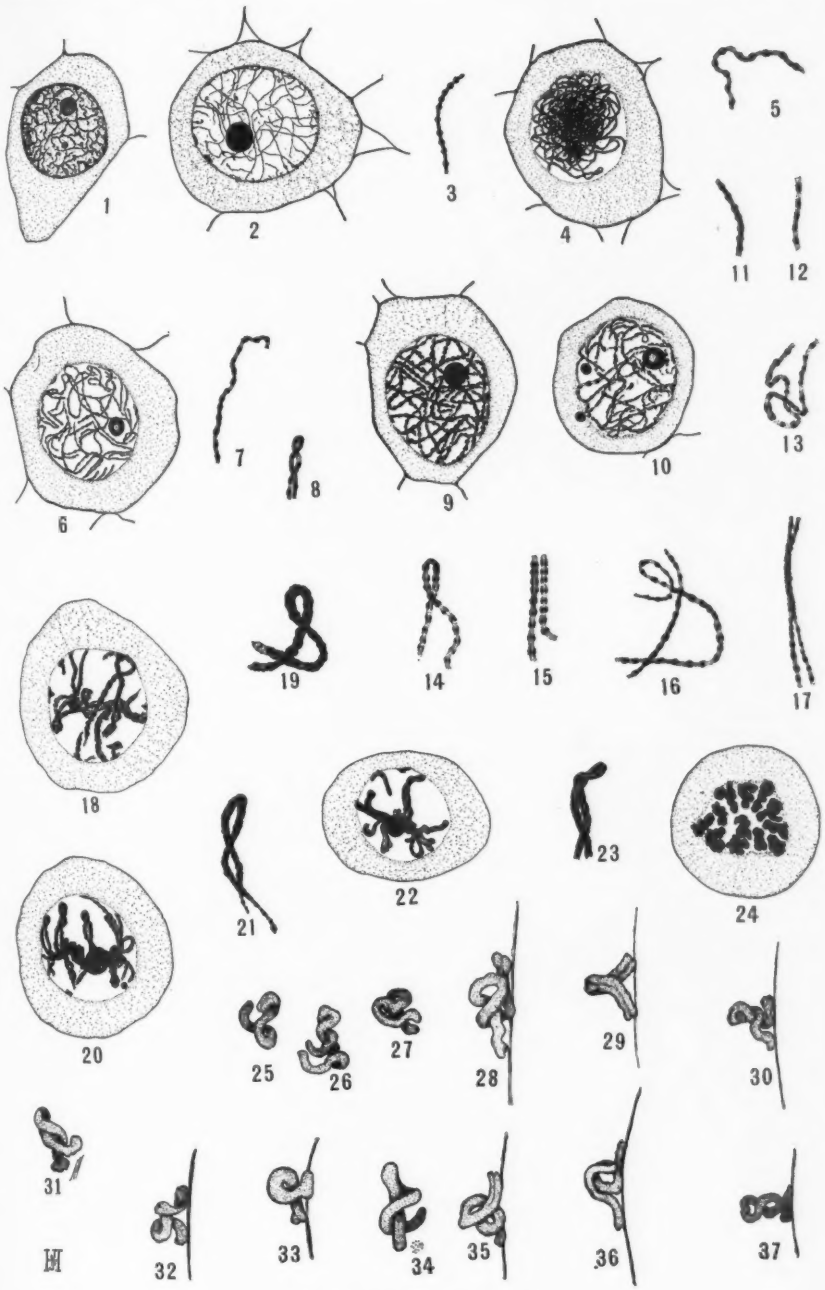
FIG. 38. Mother star, showing position of chromosomes. Micronucleoli in the cytoplasm.

FIGS. 39–42. Chromosomes from mother-star stage, showing mode of separation of limbs of loops.

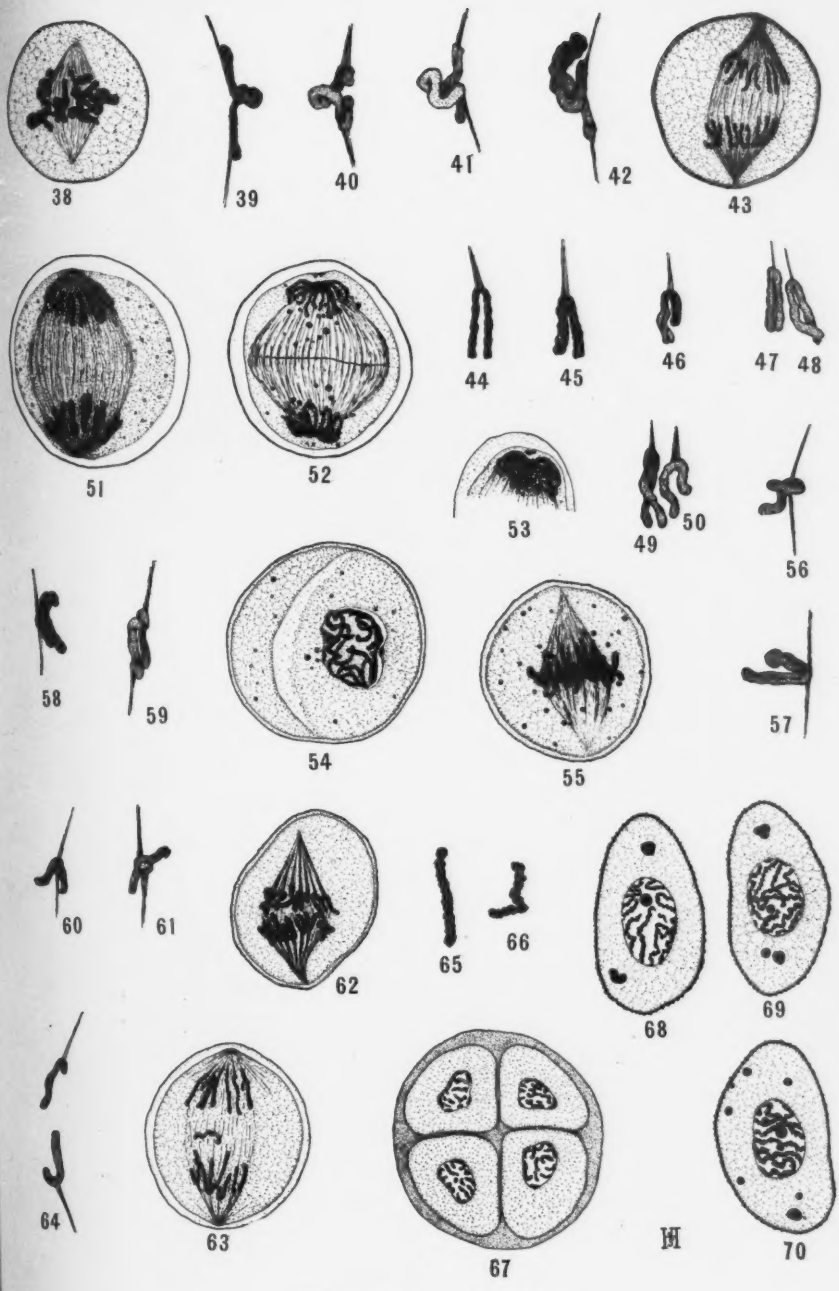
FIG. 43. Microsporocyte with daughter stars.

FIGS. 44–50. Daughter chromosomes from the daughter star, showing character of of the loops.

FIG. 51. Daughter-star stage, with micronucleoli in the cytoplasm.



SCHAFFNER on LILIUM



SCHAFFNER on LILIUM

FIG. 52. Loose daughter-skein stage with micronucleoli collected below the open ends of the chromosomes.

FIG. 53. End of loose daughter-skein stage, with micronucleoli collected among the chromosomes.

FIG. 54. Beginning of second division, with chromatin loops in the nucleus and micronucleoli in the cytoplasm.

FIG. 55. Mother star of the second division, showing characteristic appearance of the chromosomes, with micronucleoli in the cytoplasm.

FIGS. 56, 57. Single chromosomes from the mother-star stage, showing position on the spindle threads.

FIGS. 58–61. Chromosomes from the metakinesis stage, showing the nature of the longitudinal splitting.

FIG. 62. End of metakinesis stage.

FIG. 63. Daughter-star stage of second division.

FIG. 64. A pair of daughter chromosomes from the daughter-star stage of the second division.

FIGS. 65, 66. Pieces of chromosomes at the end of the second division, showing a single row of irregular chromatin granules.

FIG. 67. Tetrad at end of second division.

FIG. 68. Microspore at beginning of germination, with two large nucleoli in the cytoplasm.

FIG. 69. Same stage as the preceding, showing three nucleoli in the cytoplasm.

FIG. 70. Microspore in germination stage, showing a number of nucleoli in the cytoplasm.

CYTOLOGICAL STUDIES ON THE ENTOMOPHTHOREAE.

I. THE MORPHOLOGY AND DEVELOPMENT OF EMPUSA.[1]

EDGAR W. OLIVE.

(WITH PLATES XIV AND XV)

THE general development of the Entomophthoreae and the external morphology of its various members have been studied by a number of investigators. For a detailed review of the literature pertaining to the group, the reader is referred particularly to the papers of BREFELD ('71, '81, '84) and of THAXTER ('88).

COHN'S ('55) results in his classical paper on the developmental history of *Empusa muscae* have been in certain respects considerably modified by later investigation. According to him, the fly first became diseased and the fungus followed as a consequence. The first indications of the disease which COHN could find in the blood of the fly were numberless minute globular or irregularly shaped bodies, whose presence he could not explain otherwise than by the assumption of spontaneous generation (p. 334).

These bodies, according to him, grow larger, become globular or ellipsoidal, and finally grow into the filament, which, by the formation of partitions, becomes the three-celled hypha characteristic of the mature fungus and consisting of spore, stalk-cell, and root-cell. This three-celled character of the hyphae of *Empusa muscae* was disproved, however, the next year by LEBERT ('56) and later by BREFELD and others.

Every investigator who has attempted to infect insects artificially has testified to the difficulties which he has encountered. COHN ('55, p. 342), in speaking of his own lack of success, wisely emphasizes the caution which should characterize such experiments, noting that one should be certain that the insects experimented with are not already stricken with the disease, a more difficult task than would at first appear.

[1] I am under obligation to the Carnegie Institution of Washington for grants, which have rendered possible this investigation.

BREFELD ('70, '71, '77), however, was successful in transmitting the parasite through external inoculation of spores, and he found that in the case of *Empusa muscae* infection took place only through the thinner whitish parts of the skin on the under side of the fly's body; whereas in another species, *E. sphaerosperma*, the infected hyphae gained an entrance at any part of the skin of the cabbage larva. This author has contributed more on this point than any other, in that he was able to germinate the conidia in artificial media as well as on the surface of the insect body, and to find with the microscope the germinating hyphae actually boring through the skin of the host. In his series of articles, BREFELD has described the complete course of development of two species of Empusa, *E. muscae* Cohn and *E. sphaerosperma* Fres., which furnish quite different types of vegetative growth. According to him, in *E. muscae* there are formed from those germ-tubes which have penetrated into the body-cavity of the insect numerous detached non-nucleate cells, which reproduce by repeated yeast-like sprouting, and which grow within the fat-bodies of the host. At a certain advanced stage of the development of the fungus, the reproduction of the cells by budding ceases, and each grows at one or both ends into a long unbranched tube, which grows through the body-wall and produces at its external end a single conidium. In the other species, *E. sphaerosperma*, BREFELD found that the germ-tube produces, on the other hand, a copiously branching mycelium with many cross-partitions, which finally fills the body-cavity of the host. At the end of the vegetative period, this mycelium sends out hyphae which grow to the surface, branch digitately, and finally produce acrogenously at each ultimate end a single conidium.

Empusa sphaerosperma further differs from *E. muscae* in producing resting spores. COHN ('55, p. 343) had already suggested, since he could not make the conidiospores of *E. muscae* germinate, that perhaps the conidia themselves required a year of rest. But BREFELD ('70, '71), proved conclusively that the spores of this form were short-lived, living only for a few days; hence his first suggestion in regard to the puzzling question as to the wintering of such a species was that this form was probably heteroecious, and that resting spores were produced in some other host. Later, however ('84, p. 68), he seems

inclined to believe that the disease is continued over winter on flies in warmer regions, and that it migrates northward with the insects on the return of summer; the fallacy of which theory THAXTER has pointed out.

THAXTER ('88) in his account of the Entomophthoreae of the United States, gives the results of morphological studies based on a considerable number of new as well as old forms. This author disagrees with BREFELD in regard to certain important points. In the first place, he maintains that the vegetative growth in *E. sphaerosperma* is not filamentous in all cases, as is stated by BREFELD, and he appears to be inclined to think that both the filamentous mycelium and the broken-up, budding segments may occur in the same form under different conditions. He asserts that the usual multiplication of the hyphae is not by branching and continuous growth but by the formation of "hyphal bodies," which "consist of short thick fragments, of very varied size and shape, that are continually reproduced by budding or division, until the insect is more or less completely filled with them." But he continues further: "In cases where a direct mycelial growth follows the entrance of the hypha of germination, if indeed such instances occur, this mycelium must fall to pieces into hyphal bodies, before the commencement of growth the direct object of which is reproduction, in a fashion resembling that above described at a similar stage for Conidiobolus" (p. 140). This conception of reproduction by means of "hyphal bodies," however, for reasons that are stated later in this paper, must be abandoned, at least as a generalization.

The segments of the vegetative hyphae, or "hyphal bodies" as THAXTER terms them, under unfavorable conditions may each form a thick-walled resting chlamydospore; or, when the conditions are favorable, they may at once proceed to develop into the fructifying state. In the latter case, according to this author, each hyphal segment sends out one or two (in some species more) hyphae which develop into conidiophores. In the simplest case, a simple conidiophore grows directly to the outer air and produces a single conidium (*Empusa muscae*, e. g.). Or, the conidiophore may become compound and produce a set of conidia. Or, under very favorable conditions, "a single primary hypha may branch indefinitely, each

ultimate branch becoming a conidiophore similar to those of the more simple case just mentioned" (p. 142). A singular method of germination of the "hyphal bodies" occurs in *E. aphidis* and *E. virescens*, according to THAXTER'S observations. Spherical bodies, evidently regarded as "hyphal bodies" with highly refractive contents, germinate and send out a mass of hyphae in all directions. In this condition they are said to resemble a head of Aspergillus, although the author does not show in either of his two drawings of the phenomenon any trace or remnant of the central cells or "hyphal bodies" from which the radiating hyphae are said to arise.

The conidium is regarded by THAXTER as having a double wall, and thus is to be interpreted more properly as a simple single-spored sporangium.

MATERIALS AND METHODS.

In March 1904, the writer found in horse-dung cultures in the laboratory a small species of fly belonging to the genus *Sciara*, which was infected with an Empusa. This small fly with its attendant disease has been propagated in horse-dung cultures since that time, and many successive generations of the insect, the larval condition as well as the adult, during the year and more of its cultivation, have furnished a wealth of material for an almost complete cytological and developmental study of this species of Empusa. A number of other forms of the Entomophthoreae, most of them in the fructifying stage of their existence, have been used for comparison, but no others have as yet been traced through their entire life history. Enough has been learned, however, to show the existence of a most interesting series of distinctive variations.

My material has been killed with a variety of fixing agents, mostly with varying strengths of Flemming's chromic-acetic-osmic acid mixture. The insect body was generally cut in two or pricked to allow direct contact of the fixing fluid and the fungus hyphae in the body cavity. The material was sectioned usually 3–6 μ thick and stained with Flemming's safranin gentian-violet orange-G, or with Heidenhain's iron haematoxylin.

Six species of Empusa altogether have been thus studied. These species, determined according to the descriptions in THAXTER'S

account of the group, are as follows: *Empusa muscae* Cohn, on the common house fly; *E. culicis*, A. Braun, on a small green species of Chironomus; *Empusa* sp., on a large fly; *E. aphidis* Hoffman, on various aphides; *E. americana* Thaxter, on a blue-bottle fly; and one other species, which is the principal one studied in this paper, on the small fly, *Sciara* sp. The ovoid conidia of this last form coincide closely with the description of the conidia of *E. montana* Thaxter. They show decided differences, however, from THAXTER's drawings of this species; and from *E. ovispora* Nowakowski and *E. echinospora* Thaxter, with which the measurements of the conidia also almost coincide, the form is distinguished by the characteristic zygospores of the latter species. While it is possible that, under certain conditions unknown to me, this species on Sciara may produce zygospores, yet the fact remains that after over a year of continuous observation, I have failed to find any resting spores. It is therefore thought advisable to give a new name provisionally to this species, which will hereafter be referred to as *Empusa sciarae*.

Empusa sciarae n. sp.—Vegetative hyphae forming a branching, septate mycelium, which in advanced stages is cut up into few- (generally 3–5-) nucleate cells. Radial hyphae branched; conidiophores 3–5, bearing at each ultimate end a single ovoid uninucleate conidium with a rounded basal papilla; 12–16 × 18–25 μ. Zygospores unknown.

VEGETATIVE STAGE.

My own observations of purely vegetative stages are concerned with two forms only, *Empusa aphidis* and *E. sciarae*, both of which agree with the type described by BREFELD for *E. sphaerosperma*. In all the other species studied, the vegetative condition had ceased and conidiophores had grown out from the vegetative hyphae. As observed by BREFELD, THAXTER, and others, the insect dies at the end of the nutritive period of the fungus; but they do not seem to have emphasized the fact that living insects alone must furnish data for the study of the vegetative hyphae. After the initiation of the reproductive period and the consequent death of the insect, the radial growth of the conidiophores produces a mass of hyphae which might readily be taken, in certain instances, for a vegetative filamentous mycelial growth. The probability has suggested itself to the writer

that the fat cells, detached in the search for the fungus, must have been mistaken by some for stages in the development of Empusa; since these bodies may frequently resemble closely short hyphal segments in their fatty, granular contents, as well as in their assumption of globular or irregular shapes, which give the suggestion of budding cells. These detached fat-bodies, which prove in sections to be small aggregates of insect cells, are particularly abundant in the adult just preceding ovipositing. An easy and certain method of distinguishing the vegetative cells of Empusa from fat-bodies which occur with them in the body cavity, is to stain with a dilute methyl-green solution, acidulated with a few drops of acetic acid, when the fungus cells stand out conspicuously, distinguished by their relatively large and characteristic nuclei.

In some preliminary observations on the manner of infection of *Empusa sciarae*, I have not been able to make certain of this point, but I wish to record here some notes of interest pertaining to it. The small fly, *Sciara* sp., was sometimes accompanied, in the vessels in which the dung cultures were kept, by three other species of small flies, which hatched generally in less abundance, *Psychoda* sp., a so-called moth fly, and two other undetermined forms. None, however, other than Sciara were infected, although many times I have noted conidia stuck on the surface of the bodies of the larvae as well as of the adults of the other species. Successive generations of infected Sciara larvae as well as adults have appeared with great regularity every month or six weeks. The fly lays its eggs on the surface of the dung or on the sides of the vessels, and the young larvae, soon after hatching, crawl below the surface of the substratum. It would appear reasonable to assume that in this case infection would occur with ease in this very young condition, when the larval skin is thin and delicate, and before they had crawled below the surface of the dung, where they would obviously not be reached by shooting conidia. My preliminary unsuccessful experiments at infecting healthy adult larvae, confined for a week in bottles with diseased ones, at least suggest the possibility of infection occurring in young insects only. If the cultures in which young larvae were being nourished were kept quite moist, perhaps the majority of individuals in this stage were killed by the disease. When, however, the conditions were

drier, the insect developed frequently into the adult fly, which itself, after ovipositing, generally died of the disease.

In order to determine whether the disease could be carried from larval to adult condition, I have examined many pupae, but only in one or two instances have hyphae been found. In one case in which a young insect was struggling to get out of its pupal shell, a few Empusa hyphae were noted when the body was cut open, together with many small eggs and globular fat-bodies. When cultures were kept at some distance from infected ones, in an adjoining room, Sciara frequently developed to maturity by hundreds and died finally a natural death, evidently without infection.

Resting spores in this species, as in *E. muscae*, have not been observed; hence the puzzling question confronts us here also as to the method of wintering of the fungus. An interesting suggestion is made in this connection by the spontaneous appearance of *Empusa sciarae* in laboratory cultures as early in the spring as March, in 1904. This fact apparently still further renders useless BREFELD'S hypothesis as to the migration of the fungus north from warmer countries. The host, in this case, must have been breeding in the dung of the warm stables all through the winter, and it is quite reasonable to suppose that the short-lived fungus must have been continued at the same time on successive generations of insects. The successful cultivation of the form for more than a year in the laboratory gives additional reason for this belief. It is possible that *Empusa muscae* and such similar forms may have lost their sexual stage, because of their success in propagating the disease by means of conidia alone. It is well known that a few house-flies survive the winter by hibernation or otherwise, and it is probable that some of the individuals during the winter may continue breeding in stables or in other favorable places, and in this way carry over the disease, even in cold climates.

Living larvae of Sciara furnish beautiful material for a study of the vegetative conditions of this type of Empusa. When placed on a slide in water, a glance with the naked eye is sufficient to determine whether the larva is infected or not, since the diseased individuals are whitish in appearance, due to the presence of the more or less copious mycelium, while uninfected ones are transparent. Under the microscope, the diseased larvae show clearly the long, branched, mycelial

filaments, which in a young condition may have but few septa, while in a later vegetative stage they develop numerous septa. As the larvae crawl about over the surface of the slide, the hyphae can be seen sometimes extending the full length of the body-cavity, sometimes copiously developed in the posterior portion only, again more abundant in the anterior region. As the body parts move, or the blood flows in the cavity, the mycelium shows corresponding pulsations and movements. *Fig. 1* shows a portion of a branched vegetative hypha, taken from such a larva which was crawling just below the surface of the dung culture. Evidently the vegetative activities have here almost ceased, and the 2-, 3-, or 4-nucleate cells are about ready to send out the radial conidiophores. *Fig. 2* shows a section of a younger hypha, in which the cells contain a varying number of nuclei; while *fig. 3* represents a still younger stage, the earliest condition within the body of the host which I have succeeded in obtaining, growing in a larva in which the mycelium was composed of but a few scattered filaments. The section of the hypha, of which *fig. 3* represents only a portion, shows in the preparation twenty-two nuclei, but in the entire length, as far as traceable, not a single cross partition.

While in my own investigations of the vegetative stages of *Empusa sciarae* there remains as yet a small gap, from the penetration of the infecting hypha to the production of such multinucleate mycelial filaments as are shown in *fig. 3*, I feel reasonably certain as to the method of procedure. Germinating conidia, growing in a sterilized decoction of cooked larvae, are shown in *figs. 4–9*. *Figs. 4–7* illustrate the germination of the uninucleate conidia into germ-tubes which have grown out of the liquid decoction, thus resulting in the formation at once of secondary conidia; *fig. 7* shows the beginning of the formation of a tertiary conidium. In *figs. 8* and *9* the germ-tube has grown into a hypha in which all of the protoplasm appears to be in the end cells; in the latter, sixteen apparently empty cells separate the old conidial wall from the terminal protoplasm, which still remains uninucleate.

Both BREFELD ('**71**, *figs. 5, 29, 31*) and THAXTER ('**88**, *fig. 240*) give figures showing a somewhat further advance over what I have obtained in the cultivation of conidia, in that in their forms branches

are beginning to appear. Whether an increase of the amount of protoplasm and of the number of nuclei has accompanied this branching is doubtful.

It is highly probable, therefore, that in *Empusa sciarae* the hypha which has penetrated into the body-cavity of the fly from the germinating conidium grows rapidly at the expense of the nutrient fluids in which it floats. After the protoplasm has increased in volume, the nuclei increase in number by division, and from the uninucleate condition, in the case of *Empusa sciarae*, the hypha finally becomes a multinucleate branching filament, such as is shown in *fig. 3*. Partition-walls in this form at first occur but sparingly; later, however, at the culmination of vegetative activity, septa are abundant and branching becomes more frequent. Finally the body-cavity of the insect becomes completely filled with the mycelial filaments, vegetative activity ceases, and the fructifying state begins.

Empusa aphidis furnishes a somewhat modified vegetative development. The advanced condition, which is my only source of information in this case, shows in sections branched coenocytic hyphae, which appear to be but rarely divided by septa, unlike the corresponding stage in *Empusa sciarae*. Even after the rhizoids have grown out from the under side of the body of the insect (*figs. 10, 11*), the vegetative activities appear to continue, as evidenced by the fact that the nuclei in this instance are still undergoing division. Also in *Empusa* sp. there occurs a similar prolongation of vegetative activity, since at an advanced stage even fewer cross-partitions can be found in the coenocytic mycelium (*figs. 23, 25*).

REPRODUCTIVE STAGE.

At the culmination of vegetative growth, the body-cavity of the larva appears to be completely filled with a mass of long hyphae. Toward the close of this stage, the larva crawls to the surface of the substratum or high up on the side of the culture dish; or, in the case of the adult Sciara, the fly seeks a conspicuous position, as is common with such infected insects, and death ensues with the beginning of the fructifying condition.

The initiation of the fructifying condition is marked in *Empusa sciarae* by the sudden formation of radial branches from the short

cells which make up the hyphal filaments. These branches appear to be put forth more or less simultaneously, in this species but one from each cell. The cell swells up and becomes rounded off somewhat at its ends (*fig. 13*). One or more vacuoles appear in the protoplasm and a protuberance is pushed out from one end of the cell; this grows into the radial hypha destined to become the branched conidiophore (*figs. 12, 14, 15*). The two forces, the swelling and consequent rounding off of the cells at the ends and the pushing out of the branch, combine to split the partition-walls between the cells, thus causing the hyphae to become easily broken up into one-celled segments, the "hyphal bodies" of THAXTER (*figs. 1, 12*). I have seen similar hyphal segments, forming in these instances also the origin of the radial conidiophores, as well in my preparations of *E. culicis* and *E. americana;* but other species promise interesting variations from this common type of pre-reproductive development. In *Empusa* sp. and *E. aphidis*, for example, the vegetative hyphae remain, up to the very initiation of the reproductive stage, either unicellular, or at least with cross-partitions at only rare intervals. In such instances, therefore, no breaking up into "hyphal bodies" occurs; but the vegetative hyphae appear to grow out directly into the conidiophores.

As BREFELD and THAXTER have pointed out, the first hyphae to appear in the external growth of the fungus form the rhizoids, by means of which the host is fastened firmly to the substratum. In the common house-fly, the host is attached by means of its proboscis. In *E. sphaerosperma*, according to BREFELD, bundles of rhizoids break out more or less irregularly from the under side of the body of the insect and attach it to the substratum. According to THAXTER, these rhizoidal hyphae may branch, and may terminate in a kind of expanded sucker, which apparently secretes a viscous substance.

In *Empusa sciarae*, rhizoids are developed more or less abundantly from the under side of the abdomen of the fly, or, in the case of the larva, from almost any point on the under side of the body. In certain forms of *E. aphidis*, groups of rhizoids break out from the under surface of the insect and form large sucker-like hold-fasts. In several instances, I have counted three of these hold-fasts from aphides parasitic on Solidago. *Fig. 10* shows one of these sucker-like bundles of rhizoids in section; and *fig. 11* a highly magnified hypha from near

the inner region of the hold-fast. The latter presents clearly the character of the typical rhizoid; thick, frequently yellowish walls, containing but a thin layer of protoplasm, which bounds externally the large vacuolar cavity that almost fills the hypha. The walls of the ordinary vegetative hyphae, on the other hand, are thin; those of the rhizoids in this case appear to have undergone a gelatinous or slimy modification, and the contents seems to be undergoing degeneration.

The growth of the conidiophores, in the case of *E. sciarae*, proceeds more slowly than that of the rhizoidal hyphae, a phenomenon which is probably due, in part at least, to the slower absorption of water by those hyphae destined to bear conidia. At any rate, the vacuoles which are formed at this time in the cells increase slowly in size, and a conidiophore arises from near the end of each cell and grows out radially, boring its way through the tissues of the host (*figs. 12, 14, 15*). As THAXTER and others have noted, the conidiophores of certain species remain simple and unbranched, as in the case of *E. muscae* (*fig. 40*); or, in other species, they may become normally septate and branched, as shown in *E. sciarae* (*fig. 16*), and *Empusa* sp. (*fig. 23*). From this fact arise most interesting cytological variations in the various conidia of these species. THAXTER has brought out clearly the variation in size, shape, etc., of the conidia of many species, but CAVARA ('99) was the first to contrast the multinucleate condition of the conidia of *Empusa muscae* with that of the uninucleate conidia of *Entomophthora Delpiniana*. Of the six species studied by me, four have uninucleate conidia (*E. sciarae, fig. 27; E. americana, figs. 36, 37; E. aphidis, figs. 42, 43;* and *Empusa* sp., *figs. 22, 26*). The conidia of *E. culicis* are normally two-, rarely three-nucleate (*figs. 31, 32*); while those of *E. muscae* have a more or less indefinite number, frequently about 15–18 (*figs. 38, 39*).[2] The more common uninucleate conidia arise primarily from the septation of the conidiophores into uninucleate segments; whereas, on the other hand, the simple conidiophore of *E. muscae* does not usually become septate except at the conidium. In the last case, therefore, all of the many nuclei of the last-formed vegetative cell, which forms the origin of the

[2] While convinced of the value of CAVARA'S suggestion as to the use of nuclear characters in the classification of the Entomophthoreae I do not think that this one character alone would justify the separation of Entomophthora from Empusa.

conidiophore, flow out into the single, large, bell-shaped conidium (*fig. 39*).

In *E. culicis*, which has binucleate conidia, septation of the conidiophore occurs, by which the protoplasm cuts itself off from behind; but, unlike the case of *E. sciarae*, I have found no branching with it, so that as a consequence, all of the binucleate protoplasm of the conidiophoric hypha flows into the one terminal conidium (*figs. 30–35*). The simple conidiophore of *E. culicis* resembles, therefore, that of *E. muscae* in being unbranched, but the origin of the conidiophores may differ in the two cases. One may find, in fact, in *E. culicis*, two sorts of "hyphal bodies;" either small cells which, like the corresponding ones of *E. muscae*, give rise to but one conidiophore; or, on the other hand, larger cells, which may give rise to several conidiophores by budding from several points simultaneously, in a similar manner to Conidiobolus. In both instances, each conidiophore remains simple and ultimately bears, terminally, the binucleate conidiospore.

In the species with uninucleate conidia, *E. sciarae*, *E. americana*, *E. aphidis*, and *Empusa* sp., the coenocytic conidiophore, as was indicated above, is cut up by septa, in a manner to be described later, into uninucleate segments (*figs. 16–21*). In all these cases, this results in a branching growth and successive abjunction of the acrogenous spores. Below the terminal cell the penultimate cell pushes out to one side, and thence bores its way to the surface of the host, where it abjoints a single uninucleate conidium (*figs. 16, 23*). From this habit in certain forms, arises a profuse system of branching, frequently of a digitate type (e. g., *Empusa* sp.) or corymbose (*E. sciarae* and others), to enable the uninucleate segments to reach the surface and to discharge their protoplasm by means of the abjointed conidia. But the last-formed vegetative cells of *E. sciarae* contain only 2–4 nuclei (*figs. 1, 12, 15*), so that, in this instance, only a correspondingly small number of branches are formed.

The process of abjection of the conidia of Empusa is apparently similar in a general way to that described for the sporangia of Pilobolus, except that in these conidia there is no gelatinous collar visible. The formation of the partition at the base of the conidium in Empusa also is quite a different process from the formation of the columella in the case of Pilobolus.

In Empusa, the vacuole which appears in the basal portion of the

cell becomes larger and larger (*fig. 27*), and a small protuberance, which has a diameter equal to about half that of the conidiophore, is pushed out from the end (*figs. 34, 41*). There is now formed at the apex of this narrowed sterigma a swelling (*figs. 27, 35, 39, 42*), which, after continued enlargement, finally receives the greater part of the protoplasm and all of the nuclear content from the basal portion. The process of cell-division, by means of which this apical conidium is cut off by a wall from the penultimate cell, will be described in some detail later, in connection with the description of cell-division in Empusa. Certain points should be noted here, however, and among them, that the term ***basidium***, as applied to the penultimate cell, although in common use in this connection, should, in my opinion, be confined to the Basidiomycetes, where, morphologically, the true basidium is a very different spore-bearing structure from the penultimate cell bearing the conidium in Empusa.

The penultimate cell forms the explosive mechanism by which the conidium is shot off. As the ring-formed wall which cuts through the base of the conidium travels progressively inward, the protoplasm passes through the narrowing opening leading from below, until at the close of abjunction, the basal cell retains only a thin parietal layer of protoplasm, but no nucleus. Continued swelling, due to the absorption of water, finally results in the bursting of the basal vesicle, thereby breaking the wall of the vesicle where it joins that of the conidium. In some forms, a ring-shaped scar is noticeable near the base of the conidium, marking the circle where the summit of the swollen basal vesicle was formerly attached. The septum which separates the conidium from the subterminal cell is at first usually pushed upward, thus resembling a columella (*figs. 28, 30, 31, 36*), but when the spore is shot off, this partition-wall reverses its former position, and in the conidium it appears as a prominent papilla (*figs. 29, 37, 43*). When the basal vesicle bursts, its contents are thrown out of the open ruptured end and frequently persists as a slimy covering about the spore, serving in this case, perhaps, the double purpose of protection against excessive evaporation and of sticking the spore to the substratum which it strikes. I have noted that the explosion, in the case of *Empusa sciarae*, sometimes throws the spore a distance of 6^{mm}; while BREFELD has recorded an even greater distance in the case of *E. muscae*, in which the spores are said to be sent as far as $2–3^{cm}$.

I have not yet completely solved satisfactorily to myself the peculiar method of abjection of the spores of my undetermined species of Empusa. I am inclined to think, however, that this method, in certain respects, is unlike that described above. In this form the conidiophores come to the surface, become cut up by septa into uninucleate segments, and proceed to branch profusely (*figs. 23, 25*). The cell terminating each branch pushes out in a peculiar manner. Instead of forming a large basal vesicle as an explosive mechanism in the usual manner, as described above, here the protoplasm appears to cut itself off from behind by means of one or more successively formed walls (*figs. 22, 24, 26*). A minimum of protoplasm seems to be lost in the process, and this cut-off protoplasm soon assumes a peculiar granular appearance. In this condition it is probably dead, for these cut-off cells appear soon to lose their turgescence. It is difficult to conceive of a forcible discharge of the spore in this instance, especially if it be true that the protoplasm of the basal cells is dead and thus incapable, through loss of turgidity, of functioning as an explosive mechanism. The process here rather seems to be that, by means of these successive abjunctions, the uninucleate spores are pushed off with but little force, and that they are probably followed out of the thick, gelatinous wall of the mother hypha by other cells pushed up from below. It may be, however, that further studies on fresh material of this species will change this impression of subterminal proliferation.

Figs. 45 and *46* represent the terminal portion of large hyphae of *E. culicis* which are destined to form resting spores; and *figs. 47* and *48*, two fully formed, thick-walled resting spores. Such hyphae as are shown in the first two figures are distinguished from conidiophores by being much larger, and, further, they contain four or five nuclei, instead of two. I have traced these hyphae in sections back to large "hyphal bodies," but I was unable to follow their complete history. Whether the thick-walled resting spores of this species are therefore true zygospores, or azygospores, as they are termed in THAXTER'S monograph, I am not prepared to say. I have not been able, however, to confirm VUILLEMIN'S assertions (:00) as to the nuclear fusions in the azygospores of *Entomophthora gloeospora*.

UNIVERSITY OF WISCONSIN,
Madison.

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EXPLANATION OF PLATES XIV AND XV.

The drawings were made with the aid of an Abbé camera lucida, and for the most part with various compensating oculars combined with Zeiss 2^{mm} apochromatic obj. N. A. 1.30.

PLATE XIV.

FIGS. 1–9, *Empusa sciarae.*

FIG. 1. A freshly killed filament, fixed and stained with acetic methyl green. ×275.

FIG. 2. Section of a younger filament.

FIG. 3. Section of a still younger vegetative hypha. ×275.

FIG. 4. A germinating conidium, cultivated in a decoction of cooked larvæ, forming a secondary conidium. Killed in acetic methyl green. ×575.

FIGS. 5, 6. Conidia with still younger germ-tubes. Killed with acetic methyl green. ×575.

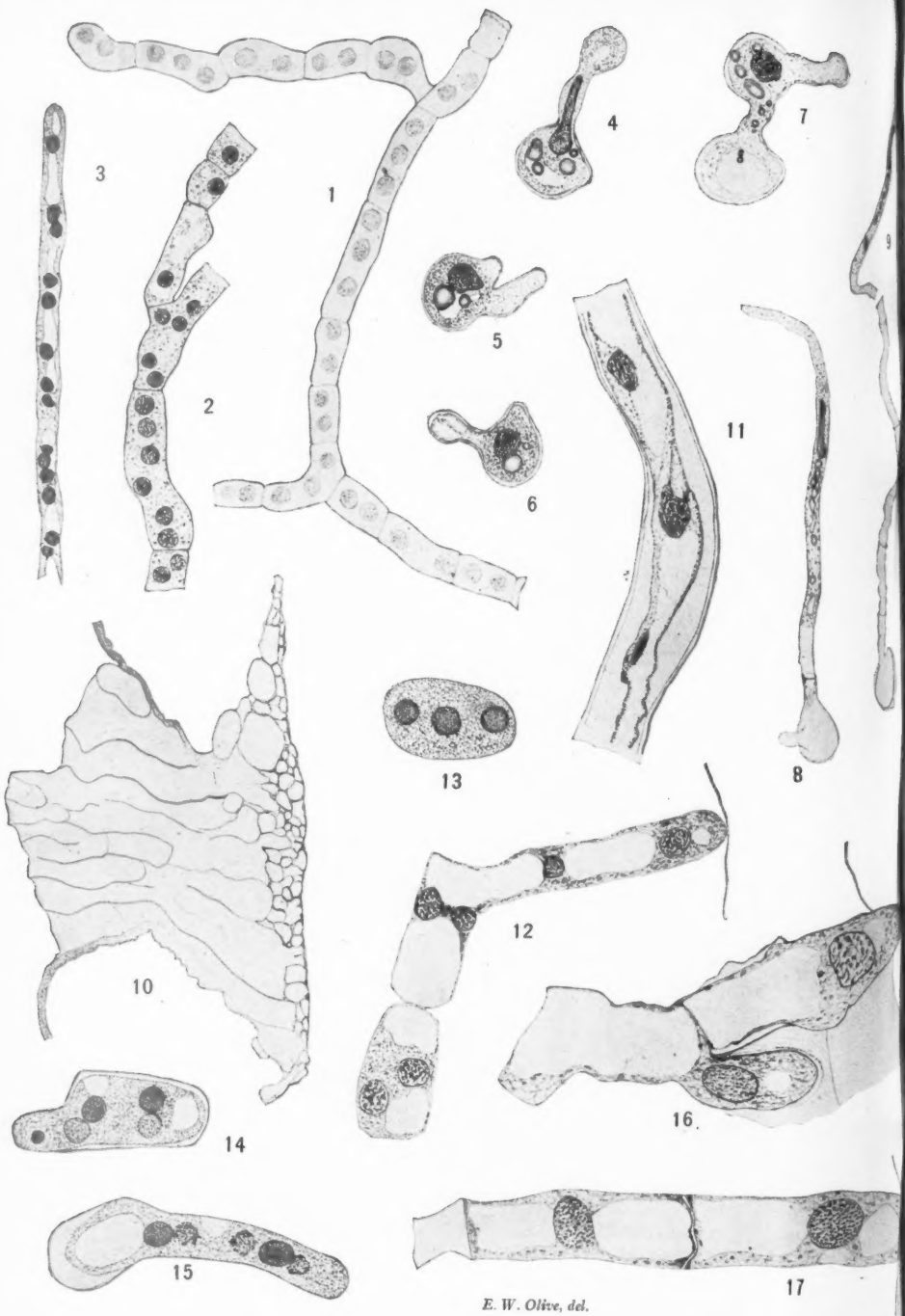
FIG. 7. A germ-tube has formed a secondary conidium, which in turn has started to form a tertiary conidium. Killed ditto. ×575.

FIGS. 8, 9. The conidia have produced long germ-tubes instead of secondary conidia. From the same culture as those above. Killed ditto.

FIGS. 10–11, *Empusa aphidis.*

FIG. 10. A cross-section of one of the sucker-like rhizoidal disks.

FIG. 11. A section of a single rhizoid. ×1080.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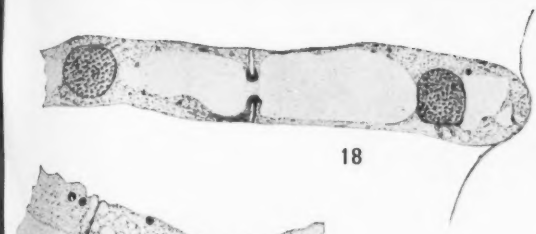
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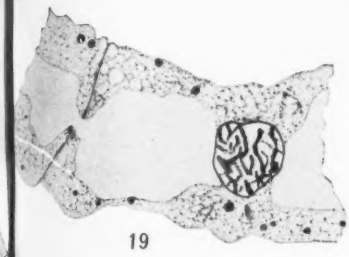
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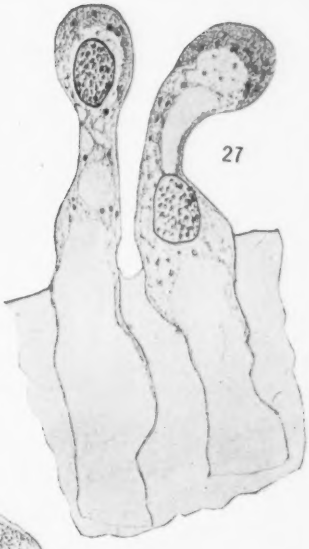
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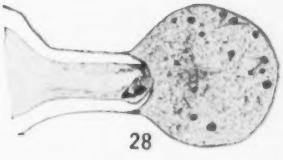
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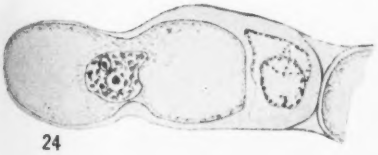
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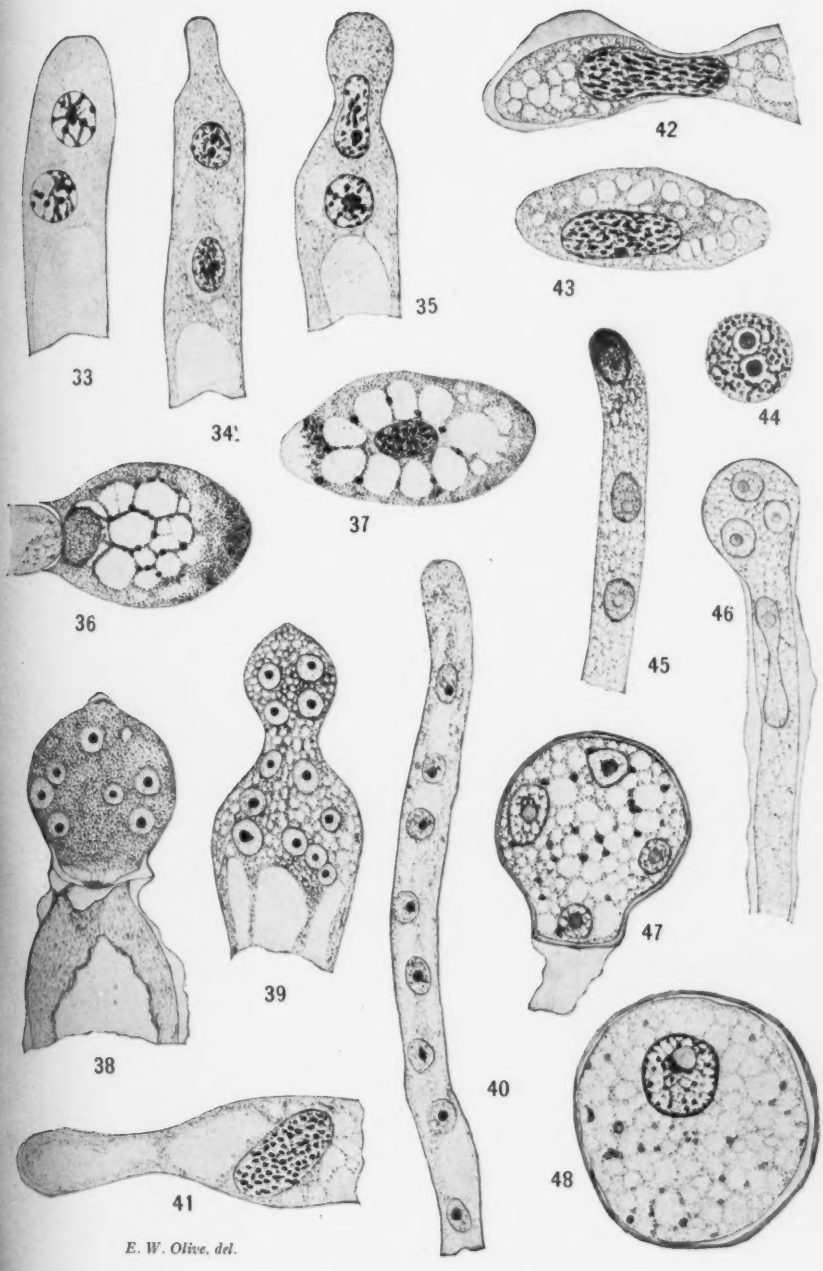

OLIVE on EMPUSA

FIGS. 12–21, *Empusa sciarae.*

FIG. 12. A section showing two cells of the old vegetative mycelium, or "hyphal bodies," from the upper one of which has grown out radially a conidiophore.

FIG. 13. A hyphal segment freshly killed with acetic methyl green. ×480.

FIG. 14. The same, showing the beginning of a conidiophore. ×480.

FIG. 15. The same, showing a still longer conidiophore. ×480.

FIG. 16. Section of a radial conidiophore which is just emerging from the epidermis of the host, showing the subterminal branching. ×1080.

FIG. 17. Section of a conidiophore showing a completed septum between the two cells. ×1080.

FIG. 18. A half-completed stage of cell-division in a conidiophore. ×1080.

FIG. 19. Cell-division in the vegetative mycelium. ×1500.

FIG. 20. An almost-completed stage of cell-division in a conidiophore; the iron-haematoxylin stain has been almost completely washed out of the preparation, except at the innermost margin of the cleft. ×1500.

FIG. 21. A poorly differentiated construction in a vegetative hypha.

FIGS. 22–24, *Empusa* sp. ?

FIG. 22. Section of a conidiophore, showing the peculiar method of the cutting off of basal cells, which apparently soon die. ×1080.

FIG. 23. Section of a conidiophore showing a subterminal branch, and also nuclei which appear to have assumed amoeboid shapes. ×1080.

FIG. 24. A similar section to that shown in fig. 22, but with only one basal cell cut off. ×1080.

FIGS. 25, 26, *Empusa* sp.

FIG. 25. End of a conidiophore, from which has been cut off two uninucleate segments. ×1080.

FIG. 26. A conidium which is apparently being pushed out of the thick, slimy wall of the mother hypha. ×575.

FIGS. 27–29, *Empusa sciarae.*

FIG. 27. A section showing two protruded terminal cells of the conidiophore, from which young conidia are in process of formation. ×1080.

FIG. 28. A section of a conidium in process of abstriction, in which the iron-haematoxylin stain has not differentiated the nucleus but has brought out some of the metachromatic bodies. ×1500.

FIG. 29. A mature conidium.

FIGS. 30–32, *Empusa culicis.* ×1500.

FIG. 30. The upper portion of the terminal cell of a conidiophore, showing at either side the cleft marking the ring-formed cleavage-furrow.

FIG. 31. A section showing a somewhat older stage, in which the cleft has almost abstricted the binucleate conidium.

FIG. 32. A rare occurrence, showing a 3-nucleate conidium.

PLATE XV.

FIGS. 33–35. *Empusa culicis.* ×1500.

FIG. 33. A thin, lightly stained section of the end of a conidiophore, showing the usual binucleate condition.

FIG. 34. An older stage, in which the sterigma is pushing out.

FIG. 35. From a still older preparation, in which the end of the sterigma has become swollen to form the binucleate conidium.

FIGS. 36, 37, *Empusa americana.* ×1500.

FIG. 36. A preparation in which the nucleus is poorly differentiated, but which shows especially clearly the vacuolar cytoplasm and the columella-like wall which finally abstricts the conidium.

FIG. 37. A mature conidiospore, showing the vacuolated cytoplasm (probably filled when living with oil-globules), the single nucleus, and the basal papilla which has been formed by the reversal of the columella.

FIGS. 38–40, *Empusa muscae.* ×1080.

FIG. 38. The partition-wall which cuts off the multinucleate conidium is here completed. The slimy enucleate protoplasm of the basal cell appears to be but little shrunken.

FIG. 39. The end of a young conidiophore, just extruded from the body of the fly, in which the conidium is in process of formation, showing the multinucleate character of the protoplasm.

FIG. 40. A portion of a conidiophore still within the body of the fly, which is growing toward an opening in one of the abdominal joints.

FIGS. 41–44, *Empusa aphidis.* ×1500.

FIG. 41. A section showing at the tip of the conidiophore a young conidium in process of formation.

FIG. 42. An older stage of conidium-formation.

FIG. 43. A mature conidium, showing the uninucleate character.

FIG. 44. A nucleus from a conidiophore, showing two nucleoles, and portions of the chromatic thread.

FIGS. 45–48, *Empusa culicis.*

FIG. 45. The end of a young azygosporic hypha. ×1080.

FIG. 46. An older condition, which shows the beginning of the formation of a resting spore. ×1080.

FIG. 47. A mature "azygospore." ×1080.

FIG. 48. A thinner section of a mature "azygospore," showing but one nucleus. Other nuclei lie below and above in the section. ×1500.

BRIEFER ARTICLES

NEW NORMAL APPLIANCES FOR USE IN PLANT PHYSIOLOGY III.[1]

(WITH TWO FIGURES)

In the two preceding articles I described several pieces of apparatus newly devised for educational work in plant physiology and explained the objects I have in view in their development. In brief I aim to provide for each of the principal physiological processes such apparatus as will be accurate in results, convenient in manipulation, and obtainable by purchase from a supply company. The company to which the manufacture has been delegated is the Bausch & Lomb Optical Co., of Rochester, N. Y. In the earlier articles I named the appliances "precision-appliances," which some, though not all of them are; they are, however, more properly *normal appliances*, which I shall henceforth call them.

VI. PHOTOSYNTHOMETER.

No fact in all the physiology of plants is more important, and hence more imperatively demands complete demonstration in botanical education, than the absorption of carbon dioxid by green plants in light, with the equivolumetric release of oxygen. There are simple ways of demonstrating the process in part, and somewhat complicated ways of demonstrating it completely; but hitherto there has been no simple method of demonstrating the entire process in one operation. This is effected, however, by the new photosynthometer described below, and illustrated in the accompanying *fig. 1*. It is called by this name for the reason that it permits photosynthesis (the quantity of the photosynthate being a function of the quantity of the gases absorbed and released) to be measured as well as demonstrated.

The instrument consists essentially of a pear-shaped plant-chamber set in a firm iron base, a graduated measuring tube with a small stop-cock at the upper end, and a connecting stopper furnished with a stop-cock of considerable bore. The total capacity of the apparatus when closed is exactly 102^{cc}, of which the 2^{cc} is for a shoot and 100^{cc} for the gases concerned. The proper amount of shoot is provided by selecting a small-leaved plant and pushing a branch down into a measuring-glass until it displaces exactly 2^{cc}

[1] Continued from BOT. GAZETTE **39**: 152. February 1905.

of water; the water-level is then noted on the stem, which is cut at this point under water, the shoot being later, when dried, placed upright in the chamber. (It hardly shows in the figure because of irregular reflections from the surface of the chamber.) Taking advantage of the fact that the shoot will carry on photosynthesis for a time in an atmosphere containing carbon dioxid in demonstrably large amount, even up to 10 per cent. or more, we add some selected percentage of that gas to the apparatus in this way. The measuring tube, with stop-cock closed, is inverted and filled with water of room-temperature, up to a figure of the graduation expressing the selected percentage, for the tube is graduated in cubic centimeters, which are, of course, percentages of the total gas capacity of the apparatus. The stopper is then placed on the tube, and its stop-cock closed; its hollow is filled with water and inverted in a pneumatic trough (or equivalent dish of water), which has been standing in the laboratory long enough to take the temperature of the air. The lower stop-cock is then opened and carbon dioxid from a generator is allowed to enter the tube, either from below or, as is most convenient, through the top of the tube. The admission of the gas may be perfectly controlled by cautious manipulation of the upper stop-cock, which is closed at the moment when the water has been wholly driven out to the bottom of the bore of the lower stop-cock, which point is held exactly at the water-level. The lower stop-cock is now closed, and the combination, which now contains exactly the desired percentage

FIG. 1.

of carbon dioxid, is lifted from the water, shaken free from all adhering drops, and placed in position on the chamber. To prevent compression, and therefore the presence of too great a quantity, of air in the chamber when the stopper is pushed into place, tiny holes (visible in the figure), matching in stopper and chamber-neck, allow free release of such pressure, and the chamber is perfectly sealed by twisting the stopper a little. The lower stop-cock is then opened, permitting the carbon dioxid of the tube to diffuse into the chamber, a process hastened by its gravitational flow. The apparatus now contains obviously 2^{cc} of plant and 100^{cc} of gas, of which a known percentage (say 5, 8, or 10) is carbon dioxid, and the remainder is air. The instrument is now placed in a bright light (not direct sunlight) for three or four hours; then the lower stop-cock is closed (as shown in the figure), shutting into the tube a sample of the gas of the chamber at the close of the experiment. The analysis of this gas can be made at leisure, and is accomplished thus. The stopper and tube are removed and placed upright in the pneumatic trough, deeply enough to allow the stopper to be taken off without admission of air to the tube. The zero mark of the tube is then brought exactly to the water surface; the upper stop-cock is cautiously opened, permitting the water to rise slowly to the zero mark, when the stop-cock is again closed, shutting into the tube exactly 10^{cc} of the gas to be analyzed. First the quantity of carbon dioxid in the tube is determined, which is accomplished by aid of a reagent tube, of the form shown at the bottom of the figure. This tube, of glass, is provided with an extension of soft rubber tubing closed by a screw clamp, and it is filled completely to the clamp with a strong solution of caustic potash. It is slipped under the water of the pneumatic trough, with the clamp closed; the air, if any, is squeezed from the upper part; and the rubber is slipped over the lower end of the measuring tube which it grips firmly. The whole is then lifted from the water, the clamp is opened, the combination is inverted and the liquid is allowed to flow back and forth several times from one tube to the other, when it will completely absorb any carbon dioxid present. The clamp is then closed, the combination is slipped again under water, and the rubber tube is pulled off, when the atmospheric pressure will instantly force up the water to the exact extent of the carbon dioxid absorbed, permitting the amount, and hence the percentage, to be read off directly. Next a determination of the percentage of oxygen present is made. This is effected by a precisely similar method, using another reagent tube containing pyrogallate of potash, freshly made up in the usual manner. A few inversions of the combination will result in absorption of all the oxygen, and the water-level in the tube when the rubber is

removed will give directly the percentage originally present. Some slender vessel may then be slipped under the tube which is removed and supported as shown by the figure.

Thus may the gas exchange in photosynthesis be demonstrated accurately, completely, logically, and conveniently.

In studying the process with beginners, the demonstration is the more striking and conclusive to them if a second instrument is set up like (and beside) the first, but covered completely from light; while even a third, like these two except that it has no plant, may advantageously be added. The comparative analyses of the gases at the close of the experiment give results leaving nothing to be desired in logical demonstration.

For all ordinary purposes, water may be used in the pneumatic trough, its slight absorption of carbon dioxid being negligible; but for great accuracy mercury may be employed. Similarly the corrections for capillarity, vapor tension, etc., may for elementary demonstration be ignored, though in precise work they would be taken into account. Temperature and barometric pressure are compensated, obviously, by the method of use of the instrument.

FIG. 2.

A much larger instrument, capable of taking large leaves, branches, or even entire potted plants, but operated upon substantially the same principle, is now in advanced preparation and will be described later.

VII. ALUMINUM SHELLS FOR TRANSPIRATION EXPERIMENTS, ETC.

In transpiration studies with potted plants it is of course necessary to eliminate evaporation from pot and soil. There are many ways of effecting this, of which the best, perhaps, is the use of a tin cup or glass jar to cover the pot, and a roof of rubber sheeting. The advantage of this method over those in which the plant is wrapped in rubber, sealed in melted wax, etc., is this,—that the rubber roof may be readily detached from the can or jar and lifted, permitting a complete change of air to the roots when the plant is watered, thus contributing greatly to the health of the underground parts.

The aluminum shells here figured (*fig.* 2) are designed to provide light, neat, clean, and easily applied covers for pots on this principle. Flower pots are now made so nearly in standard sizes that it is possible to make the shells to fit them closely, and shells will be made for the present in 3-inch, 3½-inch, 4-inch and 5-inch sizes. To hold the rubber roof tightly to the shell, a tightly-fitting band or strap of aluminum, resting in a groove just below the strengthened top of the shell, may be drawn to any desired tightness by a convenient screw-nut, shown (though dimly) on the right in the figure. The rubber roof may be attached to the plant in any of the ways ordinarily used, but I find upon the whole the best method to be the following. In the middle of a proper-sized piece of medium-thick rubber-sheeting, a hole a little smaller than the stem of the plant is made with a cork-borer, and a cut is made with scissors from this to the margin of the piece. It is then placed around the stem, the cut edges of the central hole are stretched to overlap a little, sealed together with rubber cement and held clasped until this sets. Then a line of the cement is run to the margin, sealing one edge over the other. When fully set, the margin of the rubber is clasped to the shell, all surplus material is cut away, and a very neat and perfectly tight roof remains.—W. F. GANONG, *Smith College, Northampton, Mass.*

CURRENT LITERATURE.

BOOK REVIEWS.

Bacterial diseases.

THIS quarto volume on bacterial plant diseases, by DR. ERWIN F. SMITH,[1] is a manual for plant pathologists, which, with the author's characteristic care in clearly stating details, covers the subject, from the angle at which a tube should be held during inoculation to plans for the construction of a water still and suggestions as to the proper developer for photographic plates. One of the fundamenta reasons for the abundance of half-finished work on bacterial plant diseases which is annually imposed upon the public has been the lack in all literature of just such a guide. If the admonitions which the author has scattered through the work were heeded the feeling of uncertainty which now pervades the subject in the minds of many would be promptly dissipated.

The Carnegie Institution is surely treading on dangerous ground in publishing such a manual, but if such action can be justified at all, it can be in this case. Here the need is great and the subject is so specialized as to make such a publication impossible except at a financial loss. So great was the need that a proper presentation of the material promised for the second volume could hardly be made without a considerable portion of the matter here given.

The volume is divided into three parts; 186 pages being devoted to the text, 16 to useful laboratory formulæ, and 63 to bibliography. The latter covers the general field of bacteriology, with the exception of plant diseases, this division being reserved for the second volume. The publication is well illustrated, many of the cuts showing the effect of bacteria upon plant tissue. The index covers the entire volume and will be especially useful in connection with the bibliography, which is arranged by subjects in the body of the volume.

Among the many original things in this suggestive work, the discussion of "keeping of records" and "nomenclature and classification" are of special interest. Any one who has attempted to keep an accurate history of the behavior of a plant parasite in the laboratory and in its host when the work has extended over a number of years, has felt the need of an improved system. The subject of bacterial classification has been fairly quiescent for some years, and we have been busy trying to fit the forms as they are found into MIGULA'S somewhat artificial framework. The proposition of the author to replace MIGULA'S *Pseudomonas* by *Bacterium* and send the group represented by *B. anthracis* masquerading under the name of *Aplanobacter* will come to some as a discouragement.

[1] SMITH, E. F., Bacteria in relation to plant diseases. Vol. I. 4to. pp. xii + 285. Washington: Carnegie Institution, 1905.

In spite of some minor things which seem inseparable from originality, this work is of the first quality and should be in the hands of every plant pathologist. —H. A. HARDING.

MINOR NOTICES.

Grasses of Iowa.— As a supplementary report for 1803 the Iowa Geological Survey issues part II of the *Grasses of Iowa*,[2] prepared by PAMMEL, BALL, SCRIBNER, and others. This is a descriptive and geographical study of the grasses of the state, their general and economic aspects having been treated in part I. Under each genus there is the generic description, with synonymy, a key to the species, a description of the species, often a figure, a list of localities and a map showing the distribution of each form in the state, and a statement of distribution in North America and elsewhere. There is a chapter on physiography and geology, with a map, a section on ecology, and a partial bibliography of works on grasses. The work seems very complete and should be especially serviceable to Iowa botanists. It is a pity state printers are so seldom skilful book-makers.—C. R. B.

Connecticut fungi.— The recently established natural history survey of Connecticut has begun to show results, in the publication of two bulletins listing the Hymeniales and Ustilagineae of the state. The former[3] lists 375 species in 65 genera, gives analytic keys to the genera, and illustrates the commoner species by admirable half-tones, most of which are original. The species of smuts[4] are described with lists of hosts and distribution, and notes on economic features.— C. R. B.

NOTES FOR STUDENTS.

Photosynthesis and temperature.—The interesting results of Miss MATTHAEI on temperature as a limiting factor for photosynthesis[5] have now been extended by her work in cooperation with BLACKMAN.[6] They have endeavored to interpret the quantitative variations of photosynthesis, under approximately natural conditions, in terms of the three limiting factors thereto, viz. (1) intensity of illumination, (2) temperature of leaf, (3) pressure of CO_2. When a leaf is

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[2] PAMMEL, L. H., BALL, C. R., and SCRIBNER, F. L. The grasses of Iowa. Part II, Iowa Geological Survey, supplementary report. 1903. 8vo. pp. xiv + 436, *figs. 270*. Des Moines, Iowa. 1904.

[3] WHITE, E. A., A preliminary report on the Hymeniales of Connecticut. State Geol. and N. H. Survey Bulletin 3. 8vo. pp. 81. *pls. 40*. 1905.

[4] CLINTON, G. P., The Ustilagineae or smuts of Connecticut. Idem, Bull. 5. 8vo, *pp. 45. figs. 55*. 1905.

[5] See BOT. GAZETTE **38**: 476. 1904.

[6] BLACKMAN, F. F., and MATTHAEI, G. L. C., Experimental researches in vegetable assimilation and respiration. IV. A quantitative study of carbon dioxide assimilation and leaf temperature in natural illumination. Proc. Roy. Soc. London B. **76**: 402–460. 1905.

exposed to diffuse daylight alone the amount of photosynthesis is a measure of the light, and it varies with varying light only when the amount of carbon dioxid in the atmosphere is artificially increased and the temperature is kept high. If not, photosynthesis is limited thereby and is constant though the light vary. Isolated leaves may rise more than 10° C. above a bright mercury thermometer in the sun, a result quite at variance with BROWN and ESCOMBE'S results,[7] which, however, were calculated, not observed. Further study of this point is needed.

At normal temperature leaves are not able to utilize the full amount of energy absorbed; helianthus could reach its maximum at 29° C. with about 68 per cent. full sunlight and cherry laurel with about 36 per cent. When light is the limiting factor equal intensities produce equal photosynthesis with leaves of most various structure and type. At low temperatures leaves as different as helianthus and cherry laurel have similar photosynthetic maxima, but at high temperatures these diverge. Thus at 29.5° C. the former can fix twice as much CO_2 as the latter, requiring twice as much energy to do it, of course. The essential difference in the photosynthetic activity in different leaves lies, then, in that they have different coefficients of acceleration of this function with increasing temperature. So in nature it appears that the low pressure of CO_2 (entailing slow diffusion after solution at the surfaces of the leaf cells) and the low temperatures are the serious impediments to food making.—C. R. B.

Root tubercle cultures.—Much interest has been excited during very recent years by work done in the Department of Agriculture concerning soil inoculation with various root tubercle bacteria. Widespread and rather unfortunate notoriety has been given to the work by numerous popular magazine and newspaper articles, and the impression has been gained by the public that nearly all knowledge concerning the root tubercle is to be attributed to the recent investigations conducted in the Department. This popular impression is of course erroneous. The two distinctive contributions to this subject claimed by the workers in the Department of Agriculture were that the nitrogen-gathering ability of the bacteria was heightened by new cultural methods, and that a method of transportation in dried condition, upon cotton, had been devised, whereby pure cultures could be distributed readily to farmers.

Much skepticism has existed concerning the possibility of practically heightening the nitrogen-gathering power of the bacteria, and in a recent bulletin[8] HARDING and PRUCHA claim to have demonstrated by an examination of eighteen of these cotton cultures that such packages are worthless for practical purposes since the organisms are unable to survive upon the cotton or survive in such small numbers as to be practically valueless. "Substantially identical results upon six of these packages were obtained in five separate laboratories," and the reviewer may add that similar results were obtained in his own

[7] See BOT. GAZETTE **40**: 473. 1905.

[8] HARDING, H. A., and PRUCHA, N. J., The quality of commercial cultures for legumes. N. Y. Agr. Exp. Sta. Bull. **270**: 345–385. 1905.

laboratory. The inability of the cultures to live is attributed to the method of preparation and not to any knavery upon the part of the commercial producers. A test conducted by the authors of this bulletin demonstrated the inability of the organism to survive to a satisfactory degree upon the cotton. Any intention of opposing the idea of treating the seed of legumes with living bacteria is distinctly disavowed.

It is exceedingly unfortunate that this method should have been given such wide publicity and launched as a commercial enterprise until the question as to its efficiency had been thoroughly tested.—F. L. STEVENS.

Streaming of protoplasm in mucors.—This phenomenon, although very striking and easily observed, has been little studied. The movement was noticed by WORONIN in 1866 in *Ascophanus pulcherrimus*. It was described with considerable detail in a number of species belonging to different genera by SCHRÖTER, writer of the latest account,[9] in 1897, and the conclusion was drawn that the movement was dependent upon osmotic conditions. A careful study was also made by CHARLOTTE TERNETZ in 1900, using *Ascophanus carneus*, and the conclusion was reached that it was due to the local entrance or loss of water. SCHRÖTER has confirmed and somewhat extended the work of his predecessors. For his studies he used *Mucor stol.* (as he invariably writes *M. stolonifer*) and *Phycomyces nitens*. He had better apparatus than his predecessors, and was able to arrive at some conclusions with much certainty. He found that the movement was affected only very slightly by variation in the intensity of light. The action of ether, extremes of temperature, pressure, wounds, variation in amount of carbon dioxid, was similar to that of the same agents when applied to the higher plants. The streaming is found to be due to osmotic action and transpiration and therefore does not occur in a homogeneous substratum, as for instance when the fungus is wholly submerged, or in a saturated atmosphere. The streaming is not a rotation or circulation, as in the hairs of roots and stamens and in the cells of Chara, Nitella, Vallisneria, etc., but a backward and forward-movement, in which the protoplasm, vacuoles, and nuclei participate. Occasionally the acropetal movement is somewhat balanced by a thin peripheral layer of protoplasm without vacuoles setting up a basipetal movement. Usually the movement is toward one end of the hyphae for a longer or shorter time, then stops and starts again in the opposite direction.—J. C. ARTHUR.

Germination and radium emanations.—KÖRNICKE[10] has continued his study of the effect of radium emanations on the germination of ungerminated seeds which have been exposed in both the dry and wet condition. His earlier tests were made with radium bromid contained in glass tubes. In his later study he has used a much more powerful mixture which was contained in tubes having one side of

[9] SCHRÖTER, ALFRED, Ueber Protoplasmaströmung bei Mucorineen. Flora **95**: 1–30. 1905.

[10] KÖRNICKE, M., Weitere Untersuchungen über die Wirkung von Röntgen- und Radiumstrahlen auf die Pflanzen. Ber. Deutsch. Bot. Gesells. **23**: 324–332. 1905.

aluminium, through which the emanations pass more readily. In all the trials he finds that although the germination is not prevented there is a period of retarded growth in the seedling. The elongation of the root or stem may be temporary or permanent according to the duration of the exposure. In the latter case the injured organ persists indefinitely without disorganization, but further growth of the seedling occurs in the form of secondary members. In the case of *Vicia Faba* such a condition will follow an exposure of only one hour; yet an exposure of fourteen days does not prevent germination. Since the retardation of growth occurs sooner in the root than in the stem of a given seedling, the author favors the explanation offered by other investigators, who have worked on entirely different material, that organs engaged in photosynthesis are more resistant to the emanations. The author's experiments offer no conclusive evidence on this point. Organs of seedlings from seeds exposed to emanations retain geotropic sensibility as long as they are capable of growth, the two capacities being concurrent. The same is true of heliotropic sensibility. His earlier view that radium emits enough luminosity to induce heliotropism, which was questioned by MOLISCH, is maintained. Important as these results are, it seems to the reviewer that their value would be much greater if obtained under standardized conditions.—RAYMOND H. POND.

Anatomy of Matonia.—TANSLEY and LULHAM describe the development and mature anatomical structure of a number of specimens of *Matonia pectinata* gathered by one of them on Mount Ophir in the Malay Peninsula.[11] The cotyledons in this species are bilobed as in the polypodiaceous ferns. Below the first leaf the central cylinder of the young stem consists of a rod of xylem, surrounded by parenchyma alone; later phloem appears on the outside of the stele and in the center as well. Subsequently the endodermis and "ground tissue" likewise appear within the stele, which becomes typically siphonostelic. By a process of "local dilatation of the m rgin of the leaf gaps" an internal mass of fibrovascular tissue appears, which ultimately becomes tubular and lies within the original fibrovascular tube. This inner tubular fibrovascular bundle subsequently gives off an internal tracheary strand, which may also become tubular, so that there may be in Matonia as many as three tubular bundles lying one within the other. These join each other only in the region of the nodes. The authors consider the internal fibrovascular system as a storage tissue only, since it has no direct connection with the roots, which are attached to the external cylinder, as in other ferns of this type. The views as to the morphological nature of the complex fibrovascular system of the stem in this species may be regarded as "orthodox," since the conclusion is reached that it constitutes a single stele. The hypothesis that the pith is intruded cortex is accordingly rejected, since the authors are of the opinion that the only trustworthy criterion as to the morphological value of tissues is to be derived from a study of their relation to the primary meristems of the growing point.—E. C. JEFFREY.

[11] TANSLEY, A. G., and LULHAM, Miss R. B. J., A study of the vascular system of *Matonia pectinata*. Annals of Botany **19**:476–519. *pls. 31–33*. 1905.

Chloroplasts of sun and shade plants.—LUBIMENKO[12] refuses to accept as a general law the statement formulated by TIMIRIAZEFF that the maximum photosynthesis occurs under an intensity of illumination equal to about one half that of direct insolation. By measuring the rate of photosynthesis of ombrophilous plants (Tilia and Abies) and of ombrophobous plants (Betula and Pinus) under both artificial and natural light he finds that plants differ as to the minimum insolation necessary to initiate photosynthesis. In this result the author finds basis for the conception of a specific quality of the chloroplasts. Further investigation convinces him that the curve of photochemical work is determined primarily by the specific quality of the chloroplasts and by the anatomical structure of the leaf. The influence of the latter factor is particularly evident during periods of moderate sunshine, but the potency of the former is manifest under insolation of high or low intensity.

The chloroplasts of the ombrophilous plants have greater dimensions and a sensibility almost five times greater than that of the chloroplasts of ombrophobous plants. Other tests also indicate that the pigment is more concentrated in the chloroplasts of the former.

While auxiliary data support the author's main conclusion, the chance of error through imperfect technique and ignorance of all the factors is so great that final conclusions are better withheld.—RAYMOND H. POND.

Microsporangia of Sphenopteris.—It has been suggested that the microsporangia of *Lyginodendron Oldhamium*=*Sphenopteris* (*Crossotheca*) *Höninghausii* might be found on *Telangium Scotti*. KIDSTON[13] concludes from its structure that *Telangium Scotti* cannot be the microsporangia of *Sphenopteris Höninghausii*. In no instance was organic connection between the two demonstrated.

He found a few microsporangiate pinnae referable to *Crossotheca* Zeiller in organic connection with barren pinnae of *Sphenopteris Höninghausii*. The fertile pinnule is oval, entire, and attached to the rachis by a short pedicel, which is thickened very slightly upwards before merging into the pinnule, to which it seems to be united for a short distance. The pinnules appear to be rather thick, and the vascular bundle which enters the pinnule divides into two branches, which separate slightly from each other. Each fertile lobe bears six to eight broadly lanceolate, sharply pointed, bilocular microsporangia, which in early stages bend inward, forming a small hemispherical bunch, with their apices meeting in the center, Later, the microsporangia spread outward and appear as a fringe hanging from the margin of the pinnule. The microspores are either slightly oval or circular and measure 50 to 75 μ in diameter. The walls are roughened, being covered with minute blunt points. The tri-radial ridge, marking the line of cleavage of the tetrads, is sometimes apparent.—W. J. G. LAND.

[12]LUBIMENKO, M. W., Sur la sensibilité de l'appareil chlorophyllien des plantes ombrophiles et ombrophobes. Rev. Gén. Bot. **17**: 381–415. *pls.* 10, 11. 1905.

[13]KIDSTON, R., Preliminary note on the occurrence of microsporangia in organic connection with the foliage of Lyginodendron. Proc. Roy. Soc. B **76**: 358–360, *pl. 6.* 1905.

Photic sense organs.—GUTTENBERG[14] has demonstrated that two of the ombrophilous species of his local flora have a photo-sensitive epithelium, whose response consists in maintaining the leaf in the transverse heliotropic position. The mechanism is essentially the same as was found by HABERLANDT in the so-called velvet leaves, so abundant among the ombrophilous species of the tropical hydrophytic forests. The epidermal cells function as converging lenses, so that the protoplasmic membrane which covers the floor of the cell is not uniformly illuminated. In HABERLANDT'S studies the bright spot was centrally located, but GUTTENBERG finds that for his species that it is excentric, because the papillosity is not centrally located. The result is the same in both cases, for the leaf is attuned to the distribution of interior illumination which exists when the leaf is in the transverse position. Actual tests showed that the petiole is not a factor in securing this position. Curiously enough the leaf assumes the horizontal position in diffuse light, such as occurs under the open sky on a cloudy day. In this case, however, the internal distribution of light is the reverse of that which exists under parallel rays, the central area of the floor wall being dark with a margin of increasing brightness. The stimulus apparently consists in an unequal illumination of the cell lumen.—RAYMOND H. POND.

Nature of chromatophores.—MERESCHKOWSKY[15] holds that these bodies are not organs of the plant cell and never have been, but are foreign organisms which penetrated into the colorless plasma of the cells and live there as symbionts. In support of this notion he adduces the facts that the chromatophores multiply continuously by division and do not arise *de novo;* that they are in high degree independent of the nucleus; that they are completely analogous with zoochlorellae and zooxanthellae which inhabit hydras, infusoria, etc.; that there are organisms, (e. g., the lower Cyanophyceae, such as Aphanocapsis and Microcystis) which can be considered as free-living chromatophores; that certain Cyanophyceae actually live as symbionts in the cell plasma. This theory he thinks, is the only possible explanation of the polyphyletic origin of primeval plants, which were merely amoebae and flagellates into which Cyanophyceae migrated; that the green, red, and brown Cyanophyceae account for the algae of these colors; that the plant cell-wall is due to the formation, by the symbiotic chloroplasts, of carbohydrates easily polymerized into cellulose ; which wall makes impossible the further taking of solid food and entails the quiescent nature and simple organization of plants, minus nerve, muscle, and psychic life. Here is another pyramid of theory resting on its apex.—C. R. B.

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[14]GUTTENBERG, H. R., VON, Die Lichtsinnesorgane der Laubblätter von *Adoxa Moschatellina* und *Cynocrambe prostrata*. Ber. Deutsch. Bot. Gesells. **23**:265–273. *pls. 10, 11*. 1905.

[15] MERESCHKOWSKY, C., Ueber Natur und Ursprung der Chromatophoren im Pflanzenreiche. Biol. Centralbl. **25**: 593–604. 1905.

Idioblasts of Cruciferae.—SCHWEIDLER[16] has decided to assign a systematic value to the peculiar idioblasts of the Cruciferae. The author at present reserves judgment as to their generic value, though this is expected to be established by further work. He has no doubt, however, that suborders and tribes can be accurately defined. On this basis he divides the family into three suborders. The first is characterized by the presence of idioblasts which contain chlorophyll and which are located exclusively in the mesophyll. The idioblasts of the second suborder occur in the vascular tissue and differ from those of the first group in not containing chlorophyll. The third suborder is composed of members which have both kinds of idioblasts. Just what would happen to the systematic standing of an individual so unfortunate as to have had the development of its idioblasts inhibited is certainly not for the reviewer to say, but in view of the urgent necessity of establishing systematic work upon an experimental basis rather than morphological, it is difficult to escape the conviction that more or less futility is involved in all those efforts of which this paper is an example.—RAYMOND H. POND.

Araucarineae.—A preliminary note by THOMSON[17] states that in Agathis there are many supernumerary nuclei in the pollen tube and that in Araucaria as many as thirty were counted. The pollen tube grows along the surface of the ligule for 22^{cm} or more before entering the micropyle. The anatomy of the ovule and development of the archegonia, as well as of the pollen tubes and megaspore membranes indicate that the Araucarineae occupy a very isolated position among the Coniferales.

SEWARD and FORD in an abstract of a paper[18] read before the Royal Society Dec. 14, 1905, indicate the scope of an extensive investigation of the Araucarieae. The section headings are: Introduction, distribution, diagnosis and synonymy, seedlings, root and stem anatomy, leaves and leaf traces, reproductive shoots, fossils, and phylogenetic considerations and conclusions.

The most important conclusion is that the group, unlike the Cycadales, has been derived from lycopodiaceous ancestors. The Araucarieae differ so greatly from the other Coniferales that the authors suggest the substitution of the term, *Araucariales* for *Araucarieae*.—CHARLES J. CHAMBERLAIN.

Inhibitory action.—ERRERA[19] suggests that the non-development of lateral branches or their growth in a particular position (e. g., of certain conifers) is determined by inhibitory stimuli (de nature catalysatrice si l'on veut) traversing either bark (Araucaria) or all living cells (Picea). We may conceive, he says, the apex of the stem or root as a sort of tyrant who forbids the subjacent

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[16]SCHWEIDLER, J. H., Die systematische Bedeutung der Eiweiss- oder Myrosinzellen der Cruciferen nebst Beiträgen zu ihrer anatomisch-physiologischen Kenntniss. Ber. Deutsch. Bot. Gesells. **23**:274–285. *pl.* 1905.

[17]THOMSON, R. B., Preliminary note on the Araucarineae. Science **22**:88. 1905.

[18]SEWARD, A. C., and FORD, Sibille, O., The Araucarieae, recent and extinct.

[19]ERRERA, L., Conflicts de préséance et excitations inhibitoires chez les végétaux. Mém. Soc. Roy. Bot. Belgique **42** : 27–43. 3. Aug. 1905.

branches to erect themselves or in other cases to develop, though they have the same tendency to do so as he; their geotropism or their power of growth is held in check by his own. Suppress the apex, let it die or become enfeebled, and the subjugated branches lift their heads. Several could erect themselves and take the lead, and that is sometimes observed. But ordinarily a new conflict for precedence occurs among the branches; the one nearest the apex or the most vigorous near one early asserts its supremacy and in its turn keeps its rivals at its feet. *Cf.* the independent and almost simultaneous proposal of the like idea by McCALLUM, BOT. GAZETTE **40**: 262. Oct. 1905.—C. R. B.

Ecological survey.—PETHYBRIDGE and PRAEGER[20] have added another vegetation map and ecological description to the list of vegetation surveys of the British Isles. The area discussed lies south and west of Dublin. After a historical introduction the geology, physiography, floristics, and survey methods are briefly explained. The vegetation is divided primarily into littoral, agrarian, hill-pasture, and moorland zones, and the woodlands. The zones are further subdivided into associations. These are described in detail and as far as possible related to the factors determining their occurrence. The text is accompanied by a map and five excellent plates of vegetation types. The paper will prove of especial interest to those who have followed the work of R. SMITH, W. SMITH, and LEWIS in Scotland and England.—E. N. TRANSEAU.

Alternation of generations in animals.—In criticism of CHAMBERLAIN'S paper on this subject[21] LYON[22] holds that the phylogeny of animal gametes gives no evidence of their being reduced or vestigial generations, comparable with the gametophytic generation in plants; similarity of cytological processes does not prove identity of morphological value in the two cases. He refers to the alternation in Hydrozoa, and calls attention to the earlier proposal by BEARD and MURRAY of a theory similar to CHAMBERLAIN'S. In reply CHAMBERLAIN maintains[23] that his critic fails to distinguish between a gametophytic generation and a gametophytic plant. He holds that the generations in Hydrozoa do not alternate in the botanical sense, and points out that although reduction of the gamete-bearing generation has not been proved for animals, there is strong evidence for its having occurred in plants.—M. A. CHRYSLER.

Mechanics of secretion.—PANTANELLI[24] has attempted to ascertain whether or not true secretion of enzymes occurs. He defines secretion as "the emission

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[20]PETHYBRIDGE, G. H. and PRAEGER, R. L., The vegetation of the district lying south of Dublin. Proc. Roy. Irish Acad. B. **25**: 124–180. 1905.

[21]BOT. GAZETTE **39**: 137–144. 1905.

[22]LYON, H. L., Alternation of generations in animals. Science N. S. **21**: 666–667. 1905.

[23]CHAMBERLAIN, C. J. Alternation of generations in animals. Science N. S. **22**: 208–211. 1905.

[24]PANTANELLI, E., Meccanismo di secrezione degli enzimi. Annali di Bot. **3**: 113–142. 1905.

of substances by living protoplasm, a thing possible through a self-regulated change in the condition of permeability of the plasmatic membranes such that the organism is able at pleasure to reverse it." He finds that the ferment of Roman bread and Chianti wine truly secretes invertase, by the augmentation of the permeability of the protoplasm during the period of fermentative activity. This increased permeability is general, various salts escaping more freely at the same time. In *Mucor stolonifer*, however, the emission of invertase seems to have the character of a free exit of materials from dying parts, coincident with spore formation. Whether it has a true but weak secreting power remains for further study.—C. R. B.

Respiration.— PALLADIN distinguishes[25] three sources of the respiratory CO_2 of plants: (1) nucleo-CO_2 produced by the action of enzymes, which, partly soluble, partly insoluble in expressed sap, are intimately bound up with the protoplasm; (2) stimulation-CO_2, formed by the protoplasm itself (apparently directly) under the action of stimuli; (3) oxydase-CO_2, produced by various oxidases. The process which characterizes animal and plant life consists in the excretion of nucleo-CO_2 which is formed by decomposition without the participation of atmospheric oxygen. Intramolecular respiration is a primary phenomenon, whose CO_2 is principally nucleo-CO_2 and in some cases also stimulation-CO_2. But alcoholic fermentation is no simple phenomenon, and, as KOSTYTSCHEW has shown, must be distinguished from intramolecular respiration.— C. R. B.

Apple and pear rot.— LONGYEAR[26] has published the results of his study of a rot of apples and pears due to an undescribed species of Alternaria. The same disease has so far been found in California, Colorado, and Michigan, and in Colorado is one of the most widely distributed and common diseases of apples. PADDOCK[27] was the first to call attention to it. In the case of the apple the disease attacks the fruit only, but it attacks the fruit, leaves, and young sprouts of the pear. In the apple it appears frequently first at the blossom end of the fruit and, in the case of sorts having a deep calyx-tube, a core-rot may occur. The disease may be controlled by spraying with Bordeaux mixture and plowing under or removing the diseased fruits in which the fungus is able to pass the winter.— E. MEAD WILCOX.

Reduction division.—The earliest organisms were doubtless non-sexual. In phylogeny, according to SCHAFFNER,[28] the conjugation of nucleated cells introduced

[25] PALLADIN, W., Ueber den verschiedenen Ursprung der während der Atmung der Pflanzen ausgeschiedenen Kohlensäure. Vorlaufige Mitteilung. Ber. Deutsch. Bot. Gesells. **20:** 240–247. 1905.

[26] LONGYEAR, B. O., A new apple rot. Bull. Col. Agric. Exp. Stat. 105: 1–12. *pls. 1–4.* 1905.

[27] PADDOCK, W., A new apple disease. Rept. Col. Exp. Stat. **17:** 99. 1904.

[28] SCHAFFNER, JOHN H., The nature of the reduction division and related phenomena. Ohio Naturalist **5**:331–340. 1905.

a disturbance into the life cycle and a reduction division of some kind became an inevitable accompaniment. The places at which a reduction division might, theoretically, become established in the life cycle are presented in diagram and described. A comparison between the life cycles of plants and animals is also illustrated by a diagram. SCHAFFNER believes that in the higher animals the condition appears to be similar to that found in Fucus.

The significance of a transverse division of chromosomes in interpreting the phenomena of MENDEL'S law is illustrated and discussed. — CHARLES J. CHAMBERLAIN.

Migration of salts.— In an extensive investigation of the content of nitrogen, phosphoric acid, sodium, and potassium in cultivated plants, both field and pot grown, at different periods of their development, it has been found[29] that in different plants the maximum absorption is completed at different periods, barley, spring wheat, peas, and mustard attaining this maximum at flowering, while potatoes reach it at maturity. These substances do not remain at a maximum, but in the plants other than potatoes and with the exception of phosphoric acid, migrate back, in great part, to the soil; this seems to depend on the amount of a given substance available, being greater when, say, potassium is lacking than if the appropriate materials are all supplied.— C. R. B.

Anatomy and affinity.—Another observer, SARTON, has attempted to ascertain how much help is to be had from histology in determining the validity of Jordanian species as contrasted with Linnean.[30] He studied allied plants, submitted them to cultivation under diverse conditions and then examined their structure. In some cases there were constant anatomical characters distinguishing apparently closely allied forms. On the other hand the characters were as often elusive and evidently directly adaptive. Plants long cultivated in the Jardin des Plantes and at Fontainebleau showed no anatomical differences from wild ones of the same species. Nor were there differences between the varieties having different colored flowers.—C. R. B.

Scotch moors.—The succession of plants in the moors of the Scottish southern uplands has been studied by LEWIS.[31] He finds that in all the localities visited the peat "shows a definite stratification of plant remains, indicating a swing from woodland to heath and moss, and again to woodland. In some districts, an arctic plant-bed is interposed between the lower and upper woodland beds." The vegetation changes are probably correlated with climatic changes at the

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[29] WILFARTH, H., RÖMER, H., and WIMMER, G., Ueber die Nährstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. Landw. Versuchsstat. **63**: 1–70. *pls.* 3. 1905.

[30] SARTON, A., Recherches expérimentales sur l'anatomie des plantes affines. Ann. Sci. Nat. Bot. IX. **2**: 1–115. *pls. 14.* 1905.

[31] LEWIS, F. J., The plant remains in the Scottish peat mosses. Pt. I. The Scottish southern uplands. Trans. Roy. Soc. Edin. **41**[3]:699–722. 1905.

close of the glacial period. He concludes also that the differences in the basal deposits of these moors as compared with those of the higher Cross Fell district (upon which he reported earlier) indicate the relative time of origin.— E. N. TRANSEAU.

Aberrant chromosomes.—The discovery of chromosomes of different sizes in the same nucleus in plants suggests that the attention of botanists be called to the terminology just proposed by MONTGOMERY for aberrant chromosomes in Hemiptera.[32] The term chromosomes is retained when all the chromosomes of a nucleus are alike; when they are unlike, the name *autosoma* or *autosome* is applied to a chromosome of the usual form, and *allosoma* or *allosome* to an aberrant chromosome. Unpaired allosomes are *monosomes*, and paired allosomes are *diplosomes*.—CHARLES J. CHAMBERLAIN.

Iron-algae.—After observation in the field and a study of cultures, GAIDUKOV[33] concludes that a Conferva found by him in overflow pools of the Ocka river near Rjasan accumulates iron oxid from the waters, just as other algae do calcium carbonate or silica. He thinks such iron secretion not peculiar to the bacteria, but characteristic of many organisms, not as a necessary life-process, but as an adaptive one. In the present case it seems to be protective to the akinetes, which, weighted down by the iron oxid, sink to the bottom and so pass the winter.—C. R. B.

Photosynthesis and electricity.— POLLACCI[34] announces that electric energy, when it does not exceed a given intensity, promotes very much the formation of starch in leaves, and that this effect is greater with a continuous current passing directly into the interior of the organs. Electrified leaves almost deprived of light in some cases showed starch formation, when, in the same illumination, unelectrified leaves did not. In view of the recent English work on photosynthesis these conclusions should be received with reserve.— C. R. B.

Formation of proteids.—MONTEMARTINI[35] is attacking this much investigated problem. His first paper clears the ground, records once more a good part of the extensive bibliography, and details two experiments, which lead to the conclusion that the production of proteids is greater in light than in darkness, and greater in light and air minus CO_2 than in light and normal air. Likewise it is fivefold greater in the day than in the night, and he proposes to analyze the relation of light to this result in his later experiments.—C. R. B.

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[32]MONTGOMERY, THOS. H., The terminology of aberrant chromosomes and their behavior in certain Hemiptera. Science **23**:36–38. 1906.

[33]GAIDUKOV, N. , Ueber die Eisenalge Conferva und die Eisenorganismen des Süsswassers in algemeinen. Ber. Deutsch. Bot. Gesells. **23** : 250–253. 1905.

[34]POLLACCI, G., Influenza dell' electricità sull' assimilazione clorofillina. Nota preliminare. Atti Ist. Bot. Pavia II. **11**: 7–10. 1905.

[35]MONTEMARTINI, L., Primi studi sulla formazione delle sostanze albuminoidi nelle piante. Atti R. Ist. Bot. Pavia II. **10**: 1–20. 1905.

Chemotaxis of sperms of Equisetum.—LIDFORSS,[36] to avoid anticipation by SHIBATA, has made a preliminary announcement of his discovery that the spermatozoids of Equisetum are markedly chemotactic toward solutions of malic acid especially, and also to maleic acid and its salts. Only indifference is shown to solutions of fumaric acid or of its salts. The threshold concentration of malic acid he finds to be about M/10000. Aerotaxis, which had been previously observed in the case of Marchantia spermatozoids, could not be demonstrated.—RAYMOND H. POND.

Welwitschia.—*Tumboa mirabilis* is so little known that any fresh observations are welcome. PEARSON[37] succeeded in securing material showing the development of microsporangia, microspores, megasporangia and megaspores. Observations were made upon the habit, habitat, and climatic conditions. It is probable that the plant is partially, if not wholly, insect-pollinated, and that the processes of fertilization and maturation of the seed take place more rapidly than in other gymnosperms.—CHARLES J. CHAMBERLAIN.

The cycadean integument.—This is discussed in a recent paper by Miss STOPES,[38] who takes this occasion to compare the structures of the cycad ovule with those of the fossil Lagenostoma. The single integument of the living cycads is regarded as a double structure representing two integuments of some ancestral form. The plane of fusion of the two integuments has been between the inner and outer layers of the stony coat, or between the stone and the outer flesh. —CHARLES J. CHAMBERLAIN.

A rust-resistant cantaloup.— BLINN[39] finds that the Pollock strain of cantaloups is resistant to the rust or blight which is a common and serious disease in the Rocky Ford district of Colorado. This resistance he found was transmitted through seed selected from resistant plants, and hence seed selection becomes a very practical method of controlling this destructive disease wherever it may occur. The disease is due to the fungus *Macrosporium cucumerinum* E. & E.— E. MEAD WILCOX.

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[36]LIDFORSS, B., Ueber die Chemotaxis der Equisetum-Spermatozoiden. Ber. Deutsch. Bot. Gesells. **23**:314–316. 1905.

[37]PEARSON, H. H. W., Some observations on *Welwitschia mirabilis* Hooker. Abstract of a communication to the Royal Society of London, Nov. 23. 1905.

[38]STOPES, MARIE C., On the double nature of the cycadean integument. Annals of Botany **19**:561–566. 1905.

[39]BLINN, P. K., A rust-resistant cantaloup. Bull. Col. Agric. Exp. Stat. 104: 1–15. *pls.* 1–10. 1905.

NEWS.

DR. JOHN W. HARSHBERGER is delivering a course of ten lectures on North American trees before the Wagner Free Institute of Science in Philadelphia.

DR. PEHR OLSSON-SEFFER has left Leland Stanford University and has accepted a position in connection with an experiment station in Mexico, devoted to the investigation of the growing of rubber plants. His address, which correspondents are requested to note, will be La Zacualpa Botanical Station, Escuintla, Chiapas, Mexico.

THE ANNOUNCEMENT for 1906 of the Lake Laboratory, maintained by the Ohio State University at Cedar Point, on Lake Erie, has been issued. The only instructor in botany for the season is Prof. MALCOLM E. STICKNEY, assistant professor of botany, Denison University. Consequently the courses are limited to one in general botany and one in ecology. The course opens June 25th, and closes August 3d.

THE MINNESOTA SEASIDE STATION on the Straits of Fuca, Vancouver Island, opens its doors for the sixth annual session, July 8, 1906. Owing to the low rates to the Pacific coast which will be in force, this promises to be an important year in the history of the Station. Those contemplating marine study and research are invited to write to Professor Conway MacMillan, University of Minnesota, Minneapolis, for the illustrated announcement of the Vancouver Island Laboratory-Camp.

DR. ALBERT SCHNEIDER has resigned his position as professor of botany, pharmacognosy, and materia medica at the California College of Pharmacy and has been appointed pathologist and physiologist of the Spreckels Sugar Company. DR. HENRY B. CAREY, formerly assistant to DR. SCHNEIDER, has been elected to fill the vacancy created by the latter's resignation. DR. SCHNEIDER is now giving his entire time to the investigation of the so-called California sugar beet blight, which has been the cause of great losses to California beet growers.

THE ANNUAL announcement of the Marine Biological Laboratory at Woods Hole, Mass., shows that the laboratories will open on July 5th, the regular courses of instruction extending from that date to August 16th. The department of botany for this year will be manned by DR. GEORGE T. MOORE, of Washington, D. C., and DR. JAMES J. WOLFE, of Trinity College, N. C. Miss LILIAN J. MACRAE will act as collector. Correspondence regarding botanical courses should be addressed to Dr. MOORE at the Cosmos Club, Washington.

THE *Association internationale des Botanistes* has shown commendable activity not only in the conduct of the *Botanisches Centralblatt* but also in arranging for a supply of pure cultures of fungi and algæ. Now it further announces a long list

of places from which it is ready to supply material for demonstration or investigation to members of the society. The list is too long to be republished, but it is evident that one can secure working materal from a wide range of localities. Correspondence relating to such material should be addressed to the secretary, Professor J. P. LOTSY, Leyden, Holland.

BEGINNING with January 1, 1906, the form of the publications, which in the past have appeared as bulletins of the Bureau of Government Laboratories in the Philippines, will be changed to a journal to be known as the *Philippine Journal of Science*. This publication will include original articles by members of the staff of the Bureau of Science, as well as scientific papers submitted for publication by other officials of the Philippine government and by individuals not officially connected therewith. The journal will include researches in botany, zoology, chemistry (including physiological chemistry), serums and prophylaxis, mineralogy, geology, paleontology, mining, and mineral resources. The journal is to review work which is being accomplished and to present such original results as are obtained. The subscription price is $5 (U. S.) per year. It will be possible to secure reprints of any particular series of the articles at reduced prices. The journal will be edited by Dr. PAUL C. FREER, director of the Bureau of Science, with Dr. RICHARD P. STRONG, chief of the biological laboratory, and Mr. H. D. MCCASKEY, chief of the division of geology and mining, as co-editors.

IN THE SUMMER of the present year a permanent Station for the study of arctic science will be established on the south coast of Disco Island in Danish West-Greenland. The cost of the foundation is defrayed by a gift from Mr. A. HOLCK, of Copenhagen, and the Danish government has promised an annual grant of 10,000 kroner ($3000) toward its maintenance. A laboratory, equipped with appliances and instruments especially for biological researches, will be attached to the Station, and for the present two work-places will be furnished for visiting naturalists. The visitors will have the free use of the instruments, traveling outfit, and library of the Station. Lodging will be free and a small fee will be charged only for board. The first visitors can be received in 1907, and notices, inviting application, will be issued in due course. A library of arctic literature is to be founded at the Station and to be made as complete as possible, but on account of the limited resources of the Station and the vastness of the literature, only a small proportion of it can be purchased. The Director of the Station, M. P. PORSILD, asks botanists to be good enough to come to its assistance by giving to this library works on arctic and antarctic nature, and especially on arctic biology. The publications of the Station will be sent in return, and the Station will be glad to render any service in its power. Up to May 1 Director PORSILD may be addressed at Copenhagen S., Denmark.